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# Contrasting predation risks presented by introduced brown trout and native common river galaxias in New Zealand streams

**Angus R. McIntosh and Colin R. Townsend**

**Abstract**: Alterations in the predation risk affecting macroinvertebrates in streams of the Taieri River system of New Zealand were assessed following the replacement of native common river galaxias (*Galaxias vulgaris*) in many sites by introduced brown trout (*Salmo trutta*). In laboratory channels, galaxias foraged on mayfly nymphs from benthic positions; they searched for prey at night but stayed in cover during the day except when attacking prey. Trout foraged from positions in the water column during day and night. There were no diel differences in foraging by galaxias but trout had higher reaction distances and consumed more during the day. No diel pattern was found in trout stomach contents in the field, probably because of a nocturnal increase in prey availability. Trout and galaxias populations in the field consumed similar biomasses of aquatic prey. However, these experiments indicate there have been subtle changes in the predation regime. Trout relied on visual cues to capture prey so predation risk was higher during the day and larger prey items were more at risk. In comparison, galaxias relied more on mechanical cues to capture prey and predation risk was similar during day and night.

**Résumé** : Nous avons évalué des modifications du risque de prédation pour les macroinvertébrés dans les cours d'eau du réseau de la rivière Taieri, en Nouvelle-Zélande, par suite du remplacement, en de nombreux endroits, du galaxiidé indigène *Galaxias vulgaris* par des truites brunes (*Salmo trutta*) introduites. En laboratoire, les galaxiidés se nourrissaient de nymphes d'éphémères, qu'elles capturaient à partir de positions benthiques; elles recherchaient activement leurs proies la nuit mais restaient à couvert pendant le jour, sauf pour attaquer les proies. Les truites recherchaient leur nourriture à partir de diverses positions dans la colonne d'eau, le jour comme la nuit. Il n'y avait pas de différences nycthémérales dans la recherche de nourriture chez les galaxiidés, mais les truites présentaient une plus grande distance de réaction et consommaient davantage pendant le jour. On n'a pas observé de schéma nycthéméral dans le contenu de l'estomac des truites sur le terrain, ce qui est probablement dû à une augmentation de la disponibilité des proies la nuit. Sur le terrain, les populations de truites et de galaxiidés consommaient des biomasses similaires de proies aquatiques. Nos expériences indiquent toutefois qu'il y a eu des changements subtils dans le régime de prédation. Les truites se servaient d'indices visuels pour capturer les proies, de sorte que le risque de prédation était plus élevé le jour, et que les proies de plus grande taille étaient davantage menacées. Par contre, les galaxiidés utilisaient davantage d'indices mécaniques pour capturer les proies, et le risque de prédation était semblable le jour et la nuit.
[Traduit par la Rédaction]

## Introduction

Introductions of exotic fishes have had impacts on their recipient communities ranging from multiple extinctions to subtle changes in community structure and function. Such impacts have often involved a change in the predation regime that affects lower trophic levels (Zaret and Paine 1973; Moyle 1986; Arthington 1991; Flecker 1992). A detailed understanding of these indirect community effects is necessary if we are to be able to develop a comprehensive theory of invasion biology (Townsend 1991). Here, we assess changes in the predation regime experienced by macroinvertebrates following the introduction of brown trout (*Salmo trutta* L.) to streams in New Zealand.

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Brown trout were first introduced to New Zealand in the late 1880s and have replaced native galaxiids as the major predators in many streams (Crowl et al. 1992). For example, Townsend and Crowl (1991) found that native galaxias and brown trout seldom co-occurred in a survey of 198 sites in the Taieri River (South Island, New Zealand), where the best predictor of galaxiid presence was the number of downstream waterfalls that blocked the upstream migration of brown trout. Minns (1990) also found that native and introduced fish seldom overlapped at the site level in an examination of an extensive data base including 4229 sites throughout New Zealand.

Birds and eels remain the top predators in New Zealand streams, but the replacement of other native fish by trout is likely to have altered the predation regime affecting lower trophic levels. New Zealand has a depauperate native fish fauna (less than 30 species, McDowall 1990) that has evolved in relative isolation (McDowall 1968). It is comprised almost entirely of invertebrate carnivores; the majority belong to the family Galaxiidae (McDowall 1990), of which many are reported to be nocturnal in their foraging habits (McDowall 1990; Glova and Sagar 1989*a*, 1991; Glova et al. 1987, 1992; Halstead 1994). In comparison, *S. trutta* evolved in the northern hemisphere in streams containing many competing fish species. They are specialized for feeding visually from positions in the water column on drifting prey items, can grow to comparatively large sizes, and are highly competitive and aggressive (Allen 1951; Newman 1956; Kalleberg 1958; Chapman 1966). Investigations to date show that trout have considerable diet overlap with many native fish, but appear to have different foraging periodicities and styles (e.g., Glova and Sagar 1991; Kusabs and Swales 1991; Glova et al. 1992).

In this study we quantify differences in the predation risk experienced by macroinvertebrates in the Taieri River where brown trout have replaced common river galaxias (*Galaxias vulgaris* Stokell) as the major predator in many tributaries (Townsend and Crowl 1991). We compare the predation risks presented by the two fish species by examining stomach contents of fish sampled in the field and by analyzing foraging in laboratory stream channels on different sizes of two common mayfly nymphs (*Nesameletus* and *Deleatidium*). Prey antipredator behaviour is likely to alter their vulnerability so in our experiments we specifically try to quantify predation risk when the prey do not have a chance to respond. Our null hypotheses are that *S. trutta* and *G. vulgaris* (*i*) catch prey at similar times, (*ii*) catch prey using similar strategies, and (*iii*) consume prey at the same rate.

## Materials and methods

### Feeding experiments in stream channels

Experiments in artificial stream channels focused on the predation risk presented by common river galaxias and brown trout to two contrasting mayflies (order Ephemeroptera), *Deleatidium* spp. and *Nesameletus* spp. *Deleatidium* is a grazing leptophlebiid mayfly that is numerically dominant in many New Zealand streams (Winterbourn et al. 1981). *Nesameletus* is a grazing siphlonurid mayfly that is less abundant than *Deleatidium* but its fast and conspicuous movement is likely to make it especially vulnerable to a visually feeding predator. In contrast to *Deleatidium*, which has a dorso-ventrally flattened shape and is a slow swimmer, *Nesameletus* has a torpedo shape, is a very fast swimmer, and grows to a larger size. Species names cannot be assigned to the larvae of *Deleatidium* and the two species of *Nesameletus* cannot be reliably identified from larvae (Winterbourn and Gregson 1989).

Experiments were conducted in a set of four artificial stream channels (described in McIntosh and Townsend 1994) made from polyvinyl chloride (PVC) pipe cut longitudinally in half (diameter 35 cm and depth 17.5 cm) and connected in pairs to two reservoir tanks. Well water (14°C) was recirculated in this system, producing a depth of 13 ± 0.2 cm (mean ± SE between channels) and current velocity of $17 \pm 0.2\ \mathrm{cm \cdot s^{-1}}$ in the channels. A 1 m long experimental section was defined by a fine mesh grill (2.5 mm aperture) that stopped fish and mayflies from swimming upstream and a coarse mesh grill (7 mm aperture) at the downstream end that retained fish but allowed mayflies to move out. A piece of grey PVC pipe approximately 12 cm long was placed in the centre of each channel to provide cover for the fish and rulers were placed on the bottom to provide a scale reference. The whole section was isolated from the rest of the room by black screens that prevented human movement or light from affecting fish behaviour.

We ran eight replicate trials during both day and night in which the foraging of brown trout or common river galaxias on *Deleatidium* and *Nesameletus* was assessed. We used a different fish for each trial and trials were rotated between channels to ensure that variations between channels were not associated with any of the differences observed between fish species and times.

The experiments took place between November 1993 and January 1994 (the austral summer). Brown trout between 90 and 100 mm fork length (FL) from Sutton Stream and common river galaxias between 70 and 90 mm FL from Stony Stream (see Table 1 for descriptions of these streams) were captured by electrofishing and transferred to laboratory facilities at the University of Otago. They were held in the downstream portion of the channels for at least 3 days (adequate time for them to recover and acclimatize to the channels, Schreck et al. 1976; Woodward and Strange 1987) prior to being transferred to the experimental section 24 h before the start of a trial. Mayflies were captured from Morrisson Burn, a first-order, fishless tributary of the Water of Leith, Dunedin, and transferred to aerated aquaria (30 × 30 × 40 cm) before being used in experiments. All animals were kept under a 10 h dark : 14 h light regime, reflecting the natural regime at the time of the experiments. Mayflies were fed ad libitum on green unicellular algae grown on unglazed tiles and fish were fed ad libitum on a combination of *Nesameletus*, *Deleatidium*, mealworms, white tubificid worms, and commercial dried fish food while in the holding portion of the channels (but fish were starved for 24 h before trials while in the experimental section).

The sizes of fish used are in the larger size range of fish occurring in the streams investigated in the latter half of this study. Different sizes of trout and galaxias were used in the experiments to reflect the size differences found in the Taieri River system. Prey from a fishless stream

**Table 1.** Physical variables measured from fish sampling sites.

| | Stony Creek | Ratty Creek | Canton Creek | Sutton Stream | Deep Stream | Heeney Creek |
|---|---|---|---|---|---|---|
| Grid reference | H43<br>684089 | H43<br>687094 | H44<br>737911 | H43<br>713077 | H44<br>752995 | I42<br>916325 |
| Boulder (%) | 40 | 50 | 20 | 20 | 0 | 30 |
| Cobble (%) | 50 | 25 | 10 | 50 | 10 | 50 |
| Gravel (%) | 10 | 0 | 20 | 30 | 90 | 20 |
| Bedrock (%) | 0 | 25 | 50 | 0 | 0 | 0 |
| Mean width (m) | 3.9 | 2.0 | 5.6 | 3.0 | 2.0 | 2.5 |
| Mean depth (cm) | 23 | 19 | 25 | 15 | 14 | 12 |
| Current velocity $(\mathrm{m\cdot s^{-1}})$ | 0.86 | 0.58 | 0.43 | 0.85 | 1.1 | 0.71 |
| Elevation (m) | 805 | 810 | 470 | 520 | 350 | 240 |
| Fish density $(\mathrm{No.\cdot m^{-2}})$ | 0.52 | 2.1 | 1.29 | 0.04 | 0.02 | 0.83 |
| Fish predator | Galaxias | Galaxias | Galaxias | Trout | Trout | Trout |

**Note**: Grid references are for New Zealand Map Series 260 maps.

were used to avoid the possibility that our estimates of predation risk would be biased by particular antipredator behaviours of the prey associated with the presence of either of the fish predators.

Day trials were run between 09:00 and 13:00 (New Zealand daylight saving time) and night trials were run between 21:30 (1 h after the lights went out) and 24:00. During each trial we introduced 10 small (length 5.4 ± 0.3 mm (mean ± SE), excluding cerci) *Deleatidium*, 10 large (8.2 ± 0.2 mm) *Deleatidium*, 10 small *Nesameletus* (9.5 ± 0.2 mm), and 10 large *Nesameletus* (12 ± 0.3 mm) down a feeding tube, in random order, over 40 min at a rate of approximately 1/min. The feeding tube (a 300-mm piece of 11 mm diameter pipe attached to a funnel behind the screens, with a small flow of water) released these prey items at the upstream end of the experimental section in the middle of the water column. The passage of each prey item in the experimental section and the response of the fish were recorded on videotape using an infrared light-sensitive video camera (JVC model TK 5310EG, Victor Company of Japan Ltd., Tokyo) positioned above the experimental section of the channel. Light was provided by fluorescent lights suspended above the channels during the day producing a light intensity, just below the water surface, of 1.8 $\mu\mathrm{E\cdot m^{-2}\cdot s^{-1}}$ (1 $\mu\mathrm{E\cdot m^{-2}\cdot s^{-1}} = 0.217\ \mathrm{W\cdot m^{-2}}$). At night, infrared light, which fish and mayflies were unlikely to detect (Lythgoe 1988; Heise 1992), was used. The infrared lights consisted of a 50-W bulb housed in a light-proof box behind a plastic infrared filter (Laser Innovation Ltd., Napier, New Zealand). These lights produced less than 0.02 $\mu\mathrm{E\cdot m^{-2}\cdot s^{-1}}$ of light in the visible range (<720 nm), but enabled activity in the channels to be observed on a television monitor.

From the videotapes we assessed fish behaviour by recording their position every 2 min. We described the results of their foraging in terms of the number of prey items detected, attacked, and captured. Prey behaviour at the time of detection was also recorded. Reaction distance to prey was calculated as the linear distance, viewed from above, between the prey item and the snout of the fish when the prey item was first detected. When detecting a mayfly both fish species orientated directly towards it and formed a curved or s-shaped attack form with their body so the time of detection was easily determined. Fish may have occasionally detected mayflies and not reacted. However, our observations suggested that if fish were acclimated, these behavioural characteristics were a reliable indicator of when fish first detected mayflies.

### Field survey of stomach contents

We selected three first- or second-order streams containing common river galaxias and three containing brown trout in which to sample fish stomach contents. All were tributaries of the Taieri River and were similar in their physical characteristics (Table 1). Because the taxonomic status of galaxiids in the Taieri River is under review we chose streams with the same genetic type of galaxias (R.M. Allibone, T.A. Crowl, J.M. Holmes, T.M. King, R.M. McDowall, C.R. Townsend, and G.P. Wallis, Department of Zoology, University of Otago, unpublished data).

Between 10 and 15 fish were caught by electrofishing each stream between 14:00 and 17:00 New Zealand standard time (day sample; sunset was after 18:45) and between 02:00 and 05:00 (night sample; sunrise was after 06:30). Previous studies of galaxiid and trout stomach contents (Glova and Sagar 1991; Kusabs and Swales 1991; Glova et al. 1992) indicated that samples collected at this time would enable any diel differences to be detected. Sampling took place between March 30 and April 11, 1992, soon after the austral autumn equinox during the dark phase of the moon. After fish sampling we measured the physical characteristics of each site. Depth was recorded and the percent substrate composition of gravel (5–60 mm), cobble (60–180 mm), boulder (≥180 mm), and bedrock (defined as substrate that could not be removed from the bed) were visually estimated at each site for 10 randomly placed quadrats (0.5 $\mathrm{m^2}$). Flow velocity was measured by estimating the time it took fluorescein dye (released in the middle of the water column of the thalweg) to travel the length of each site. Elevation was obtained from maps.

We electrofished in an upstream direction to avoid catching fish that had the opportunity to feed on invertebrates

**Fig. 1.** The mean proportion (± SE) of time spent by brown trout and common river galaxias under the cover of a PVC tube during the day (open bars) and the night (shaded bars) for brown trout and common river galaxias. *N* = 8.

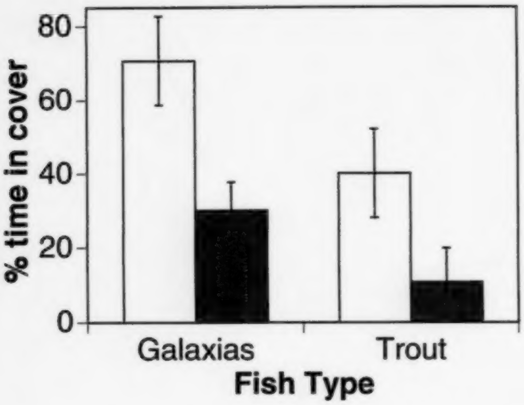
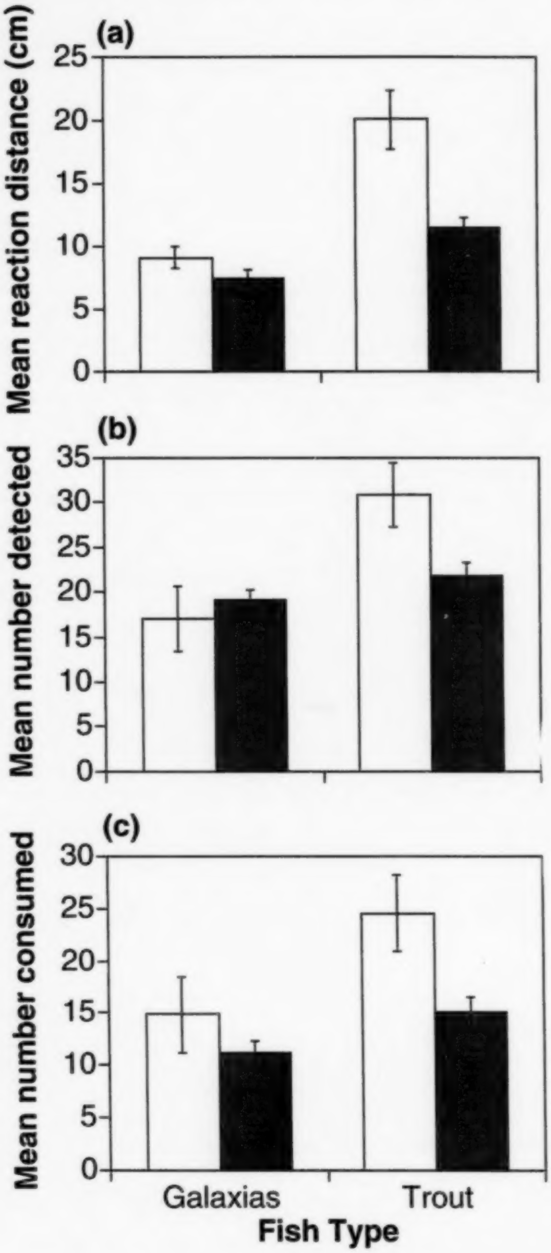


**Fig. 2.** (*a*) Mean (± SE) reaction distances for all prey types, (*b*) mean (± SE) number of prey detected per fish, and (*c*) mean (± SE) number of prey consumed per fish during the day (open bars) and night (shaded bars) in trials with common river galaxias and brown trout. *N* = 8.



disturbed by our activities. Separate (a minimum of 80 m apart), but adjacent and similar sections were fished during day and night to avoid capturing fish that had been affected by electrofishing or disturbance in the previous 12 h. Captured fish were anaesthetized and killed with a lethal dose of 2-phenoxyethanol (approximately $2.0 \times 10^{-5}$ g·L$^{-1}$) and then preserved in 10% Formalin. Fish were treated and preserved individually to prevent any regurgitated items from being lost.

In the laboratory, fish were measured (FL, to the nearest millimetre) and weighed and their stomach contents were removed. Recently eaten items were identified according to Winterbourn and Gregson (1989). All items, including digested remains that could not be identified, were later dried at 60°C for 24 h and weighed. Although different taxa will be digested at different rates we used the recently eaten items as an indication of diet composition.

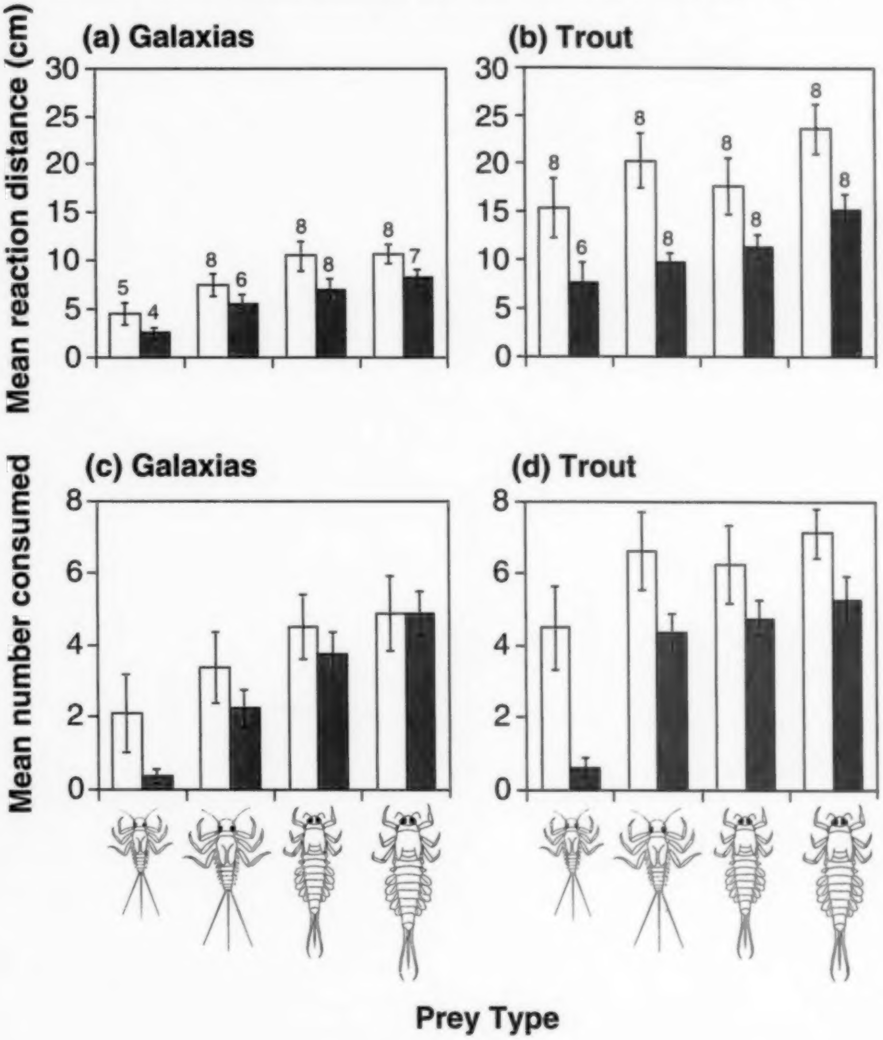
Potential differences in the physical characteristics of sites with trout and galaxias were assessed using discriminant functions analysis on the scores of principal components calculated from the physical variables. Patterns in the number of ($\ln(x + 1)$ transformed) recently eaten prey items and the biomass ($\ln(x + 1)$ transformed) of prey items were compared for the two populations and times using nested analysis of variance and covariance (Zar 1984). For these analyses a Bonferroni correction was applied to critical $p$ values ($\alpha = 0.0125$) because four tests were used to determine whether food consumption was different between the fish. The importance of major prey groups in fish diet was compared between species and times using Spearman rank correlations (Zar 1984).

## Results

### Feeding experiments in stream channels

We observed considerable differences in the behaviour of brown trout and common river galaxias in the channels. When foraging, trout maintained positions above the substratum in the water column but galaxias only left the substratum when attacking prey items. The two predators showed different diel patterns in their activity and use of cover. During the day and night trout detected and captured

**Fig. 3.** Mean (± SE) reaction distances (*a* and *b*) and mean (± SE) number consumed (*c* and *d*) of small and large *Deleatidium* (left) and small and large *Nesameletus* (right) during day (open bars) and night (shaded bars) in trials with common river galaxias (*a* and *c*) and brown trout (*b* and *d*). Numbers shown at the top of bars indicate the number of replicate fish used to calculate mean reaction distances (some types of mayfly were not detected by some fish).



prey in the same manner and spent similar amounts of time in cover (arcsin√ transformed data: $t = 1.82$, $P = 0.09$, Fig. 1), although they did tend to be more active at night. Galaxias spent most time under cover during the day ($t = 2.98$, $P = 0.011$, Fig. 1). They detected and captured prey from positions in and around the cover tube and rarely moved around inside the channel. At night this pattern changed dramatically. They spent most time moving around the channel using short movements punctuated by frequent stops. They encountered mayflies on the substratum but did not show the characteristic reaction behaviours described earlier (see Materials and methods) when approaching these mayflies. They only captured mayflies that were touched or disturbed from the benthos. We did not include these observations in our calculations of reaction distance.

Despite the behavioural differences noted above, we observed that common river galaxias had similar reaction distances ($t = 1.47$, $P = 0.17$, Fig. 2*a*), detected similar numbers of prey ($t = -0.51$, $P = 0.6$, Fig. 2*b*), and consumed similar numbers of prey ($t = 0.96$, $P = 0.37$, Fig. 2*c*) during the day and night. In comparison, trout reacted to prey at greater distances ($t = 3.33$, $P = 0.01$, Fig. 2*a*), detected more prey ($t = 2.77$, $P = 0.017$, Fig. 2*b*), and consumed more prey ($t = 2.42$, $P = 0.038$, Fig. 2*c*) during the day than the night.

**Table 2.** Factorial analysis of variance of the effect of time (day vs. night), mayfly species (*Deleatidium* vs. *Nesameletus*), and mayfly size (small vs. large) on average reaction distances (ln transformed) and total number consumed ($\ln(x + 1)$ transformed) from trials with common river galaxias and trials with brown trout.

| | df | MS | *F* | Prob. |
|---|---|---|---|---|
| **Common river galaxias** | | | | |
| Reaction distance | | | | |
| Time | 1 | 1.759 | 9.87 | 0.003 |
| Species | 1 | 6.318 | 35.44 | <0.001 |
| Size | 1 | 2.045 | 11.47 | 0.001 |
| Time × species | 1 | 0.052 | 0.29 | 0.59 |
| Time × size | 1 | 0.100 | 0.57 | 0.46 |
| Species × size | 1 | 0.839 | 4.71 | 0.03 |
| Time × species × size | 1 | 0.019 | 0.11 | 0.75 |
| Error | 46 | 0.178 | | |
| Number consumed | | | | |
| Time | 1 | 0.044 | 0.15 | 0.70 |
| Species | 1 | 11.487 | 39.97 | <0.001 |
| Size | 1 | 2.836 | 9.87 | 0.003 |
| Time × species | 1 | 1.356 | 4.72 | 0.03 |
| Time × size | 1 | 0.887 | 3.08 | 0.08 |
| Species × size | 1 | 2.037 | 7.09 | 0.01 |
| Time × species × size | 1 | 0.069 | 0.24 | 0.63 |
| Error | 56 | 0.288 | | |
| **Brown trout** | | | | |
| Reaction distance | | | | |
| Time | 1 | 5.081 | 29.64 | <0.001 |
| Species | 1 | 1.599 | 9.33 | 0.004 |
| Size | 1 | 1.698 | 9.9 | 0.003 |
| Time × species | 1 | 0.364 | 2.13 | 0.151 |
| Time × size | 1 | 0.003 | 0.02 | 0.89 |
| Species × size | 1 | 0.003 | 0.02 | 0.90 |
| Time × species × size | 1 | 0.006 | 0.04 | 0.85 |
| Error | 54 | 0.171 | | |
| Number consumed | | | | |
| Time | 1 | 2.50 | 16.56 | <0.001 |
| Species | 1 | 3.76 | 24.88 | <0.001 |
| Size | 1 | 2.54 | 16.84 | <0.001 |
| Time × species | 1 | 0.58 | 3.85 | 0.06 |
| Time × size | 1 | 0.52 | 3.42 | 0.07 |
| Species × size | 1 | 1.39 | 9.22 | 0.004 |
| Time × species × size | 1 | 0.67 | 4.46 | 0.04 |
| Error | 56 | 0.15 | | |

**Note**: Sample sizes for reaction distances (shown in Fig. 3) were uneven because some prey types were not detected in some trials. Adjusted mean squares (MS) for reaction distances were used to allow for these uneven sample sizes. df, degrees of freedom; *F*, *F* ratio; Prob., probability.

**Table 3.** Factorial analysis of variance for the effects of mayfly behaviour and time on the number of ($\ln(x + 1)$ transformed) *Deleatidium* and *Nesameletus* mayflies detected by common river galaxias and brown trout.

| | df | MS | *F* | Prob. |
|---|---|---|---|---|
| **Common river galaxias** | | | | |
| *Deleatidium* | | | | |
| Behaviour | 2 | 5.964 | 19.0 | <0.001 |
| Time | 1 | 0.046 | 0.15 | 0.703 |
| Behaviour × time | 2 | 0.578 | 1.84 | 0.171 |
| Error | 42 | 0.314 | | |
| *Nesameletus* | | | | |
| Behaviour | 3 | 2.200 | 8.24 | <0.001 |
| Time | 1 | 2.432 | 9.11 | 0.004 |
| Behaviour × time | 3 | 1.244 | 4.66 | 0.006 |
| Error | 56 | 0.267 | | |
| **Brown trout** | | | | |
| *Deleatidium* | | | | |
| Behaviour | 2 | 15.511 | 68.03 | <0.001 |
| Time | 1 | 0.017 | 0.07 | 0.79 |
| Behaviour × time | 2 | 1.091 | 4.79 | 0.01 |
| Error | 42 | 0.228 | | |
| *Nesameletus* | | | | |
| Behaviour | 3 | 6.46 | 22.53 | <0.001 |
| Time | 1 | 0.011 | 0.04 | 0.85 |
| Behaviour × time | 3 | 1.853 | 6.46 | 0.001 |
| Error | 56 | 0.287 | | |

**Note**: Behaviours are described in Fig. 5. Abbreviations are as in Table 2.

For each type and size of mayfly, the reaction distances of galaxias were all higher during the day (Fig. 3). These reaction distances were significantly affected by mayfly size, species, and time of day (Table 2). There was also an interaction between species and size, indicating that size affects reaction distances by galaxias for *Deleatidium* but not for *Nesameletus* (Fig. 3*a*). Although the reaction distances of galaxias were affected by time, consumption was not different during day and night, but mayfly species and size did influence consumption (Table 2). There were interactions between mayfly species and time and between mayfly species and size (Table 2), indicating that time and mayfly size affected consumption by galaxias of *Deleatidium* but not of *Nesameletus* (Fig. 3*c*).

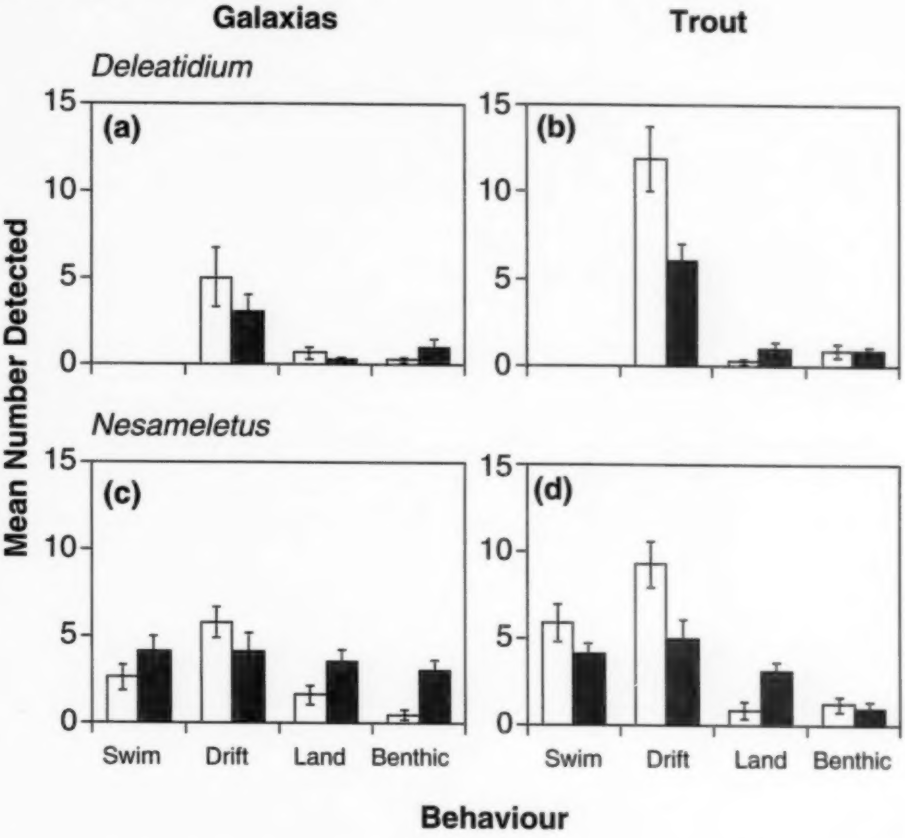
Diel differences in the foraging of trout were very prominent for different species and sizes of mayflies (Figs. 3*b* and 3*d*). Analysis of variance (Table 2) indicated that the reaction distances of trout were influenced by mayfly size, species, and time. These three main effects were important in determining the number consumed (Table 2) but there was also a significant three-way interaction. This reflects the lower numbers of small *Deleatidium* captured, especially during the night, in comparison to other prey types (Fig. 3*d*).

Our observations of mayfly behaviours at the time of detection (Fig. 4) indicate that mayflies were detected by both trout and galaxias most often while in the water column (either swimming or drifting), as indicated by the significant behaviour effect for all predator and prey combinations (Table 3).

### Field survey of stomach contents

All six streams were situated in catchments containing mixed pasture and tussock grasses. There were no consistent

**Fig. 4.** Mean (± SE) number of *Deleatidium* (*a* and *b*) and *Nesameletus* (*c* and *d*) mayflies detected by common river galaxias (*a* and *c*) and brown trout (*b* and *d*) while swimming in the water column (swim), while drifting in the water column (drift), <10 s after landing on the substratum (land), and while on the substratum (benthic) during the day (open bars) and the night (shaded bars). $N = 8$.



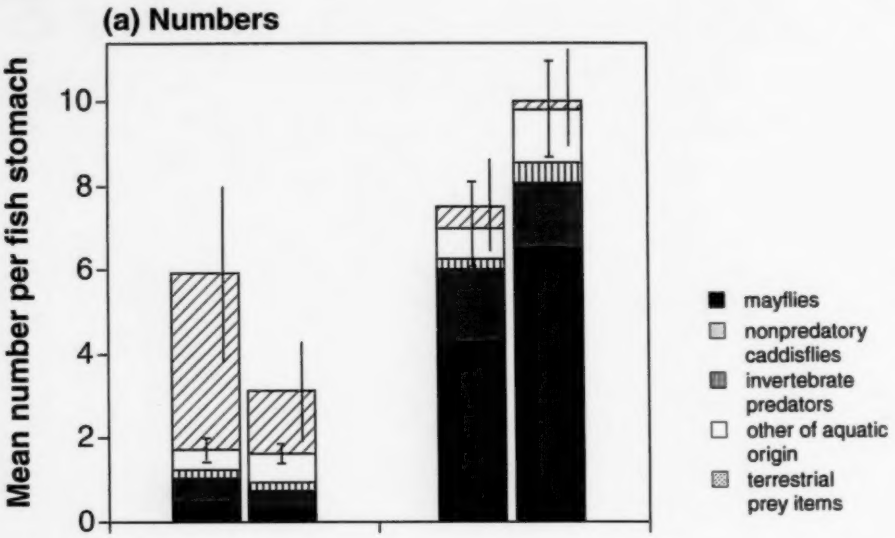
differences in the physical characteristics of the three galaxias streams and three trout streams sampled (Table 1). Discriminant functions analysis, with cross validation, could not correctly classify the sites according to their predation regime using the scores from three principal components that together accounted for 93% of the variance in the physical variables.

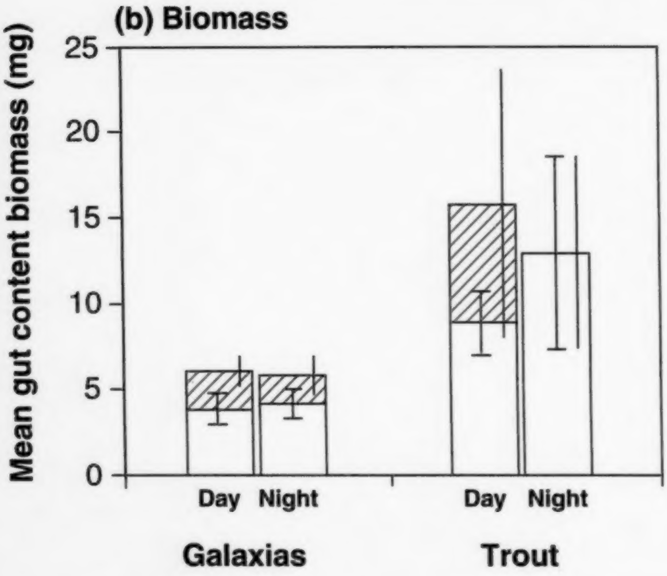
The sampled common river galaxias were smaller than the trout (71.63 ± 1.22 and 83.27 ± 2.07 mm FL, respectively) and fish densities were higher in the streams with common river galaxias than in those with trout (1.3 ± 0.46 and 0.46 ± 0.27 $m^{-2}$, respectively).

Trout stomachs contained a larger number of recently eaten prey items (Fig. 5*a*, Table 4). There were similar numbers of aquatic prey items in galaxias stomachs during the day and night but more in trout stomachs at night (Fig. 5*a*). However, there was no significant interaction between time and predator type (Table 4). The combined number of aquatic and terrestrial prey was higher in galaxias stomachs during the day but higher in trout stomachs at night so there was a near significant interaction between predator and time (Table 4). Figure 5*b* shows that the biomasses of aquatic prey and terrestrial prey were similar in common river galaxias guts during the day and night. Brown trout had generally higher gut biomass with more terrestrial biomass during the day (Fig. 5*b*). However, when these data were analyzed with analysis of covariance the covariate, fish weight, was highly significant and there were no significant differences between predators or times (Table 4).

Both fish species fed on a wide range of aquatic prey items (see Appendix) and their diets overlapped considerably; 59% of the aquatic taxa identified were found in the diet of both fish species. We considered diet in terms of five classes: mayflies, nonpredatory caddisflies, invertebrate predators, others, and terrestrial items (see the appendix for classifications). Comparison of the number of prey in these classifications (Fig. 5*a*) showed that the distribution of different prey types was similar during the day and night in both trout and galaxias guts (Spearman rank correlations ($r_s$) = 0.82 and 0.9, respectively). However, the common river galaxias and brown trout consumed prey types in different proportions. The number of prey items in each category

**Fig. 5.** (*a*) Mean number of recently eaten prey items per fish found in stomachs of galaxias and trout during the day and the night for five classes of prey: mayflies, nonpredatory caddisflies, invertebrate predators, others of aquatic origin, and terrestrial prey items. (*b*) Mean prey dry weight (mg) in galaxias and trout stomachs. Hatched bars indicate biomass of terrestrial prey. In both *a* and *b*, centered error bars indicate standard errors for the means of aquatic prey and error bars to the right of bars indicate standard errors for means of all prey.


(a) Numbers


(b) Biomass

of galaxias diet during the day and night was uncorrelated with day ($r_s = 0.2$ and $r_s = 0.0$, respectively) and night ($r_s = -0.2$ and $r_s = -0.4$, respectively) diet of trout. Figure 5*a* shows that the trout diet included a smaller number of nonpredatory caddisflies and terrestrial items, but a larger number of mayflies than the galaxias diet.

## Discussion

The introduction of an exotic predator can be expected to have an influence on the recipient prey community if it significantly alters the predation regime. Given the differences in their natural histories, the replacement of common river

**Table 4.** Results from nested analysis of variance for total number of recently eaten prey items per fish and number of recently eaten aquatic prey items per fish, and from nested analysis of covariance for total gut dry weight (mg) and dry weight of aquatic items (mg) with fish dry weight (g) as the covariate.

| | df | Adjusted MS | *F* | Prob. |
|---|---|---|---|---|
| Total numbers per fish | | | | |
| Predator | 1 | 51.18 | 20.36 | 0.011 |
| Time | 1 | 0.75 | 1.56 | 0.21 |
| Stream(predator) | 4 | 2.51 | 5.21 | 0.001 |
| Predator × time | 1 | 1.15 | 2.38 | 0.13 |
| Time × stream(predator) | 4 | 0.91 | 1.89 | 0.11 |
| Error | 155 | 0.48 | | |
| Total | 166 | | | |
| Aquatic numbers per fish | | | | |
| Predator | 1 | 30.03 | 11.88 | 0.026 |
| Time | 1 | 0.008 | 0.01 | 0.92 |
| Stream(predator) | 4 | 2.53 | 3.86 | 0.005 |
| Predator × time | 1 | 3.32 | 5.08 | 0.026 |
| Time × stream(predator) | 4 | 1.52 | 2.32 | 0.06 |
| Error | 155 | 0.65 | | |
| Total | 166 | | | |
| Total biomass (mg) | | | | |
| Fish weight | 1 | 18 948.8 | 59.71 | <0.001 |
| Predator | 1 | 151.6 | 0.156 | 0.713 |
| Time | 1 | 267.0 | 0.84 | 0.36 |
| Stream(predator) | 4 | 972.1 | 3.06 | 0.02 |
| Predator × time | 1 | 657.5 | 2.07 | 0.15 |
| Time × stream(predator) | 4 | 397.7 | 1.25 | 0.29 |
| Error | 154 | 317.3 | | |
| Total | 166 | | | |
| Aquatic biomass (mg) | | | | |
| Fish weight | 1 | 37.31 | 49.16 | <0.001 |
| Predator | 1 | 0.0006 | 0.008 | 0.96 |
| Time | 1 | 0.31 | 0.4 | 0.53 |
| Stream(predator) | 4 | 0.08 | 0.1 | 0.98 |
| Predator × time | 1 | 1.17 | 1.55 | 0.22 |
| Time × stream(predator) | 4 | 2.48 | 3.27 | 0.01 |
| Error | 154 | 0.76 | | |
| Total | 166 | | | |

**Note**: The number of fish examined for each sample was as follows (stream: day, night): Stony Creek: 15, 15; Ratty Creek: 15, 15; Canton Creek: 10, 12 (common river galaxias sites); Sutton Stream: 11, 15; Deep Stream: 14, 15; Heeney Creek: 14, 16 (brown trout sites). Data were $\ln(x + 1)$ transformed where necessary and compared between times, predator type, and streams (nested within predator type). Time and predator type were fixed variables but stream was random and the stream(predator type) term was used to test the main effect of predator type. For these tests $\alpha = 0.0125$. df, degrees of freedom; Adjusted MS, mean square adjusted for uneven sample sizes; *F*, *F* ratio; Prob., probability.

galaxias by brown trout in many streams in the Taieri River system might have been expected to alter the predation regimes. We observed considerable differences in the predation risk presented by the two predators in our foraging experiments. Common river galaxias spent most time during the day in cover and were active at night, but consumed similar numbers of prey during day and night. Brown trout were active during the day and night but consumed more prey during the day.

Similar patterns of nocturnal activity in *G. vulgaris* were recorded by Cadwallader (1975*a*) and McIntosh and Townsend (1994). The significance of nocturnal activity, a common feature of many of New Zealand's native fish (McDowall 1990), is unclear but may relate to the avoidance of avian predators (but see Glova et al. 1987).

Galaxias used a sit and wait strategy during the day, but actively pursued prey during the night. Reaction distances for individual prey types were higher during the day, but because galaxias detected more small *Deleatidium* during the day, mean reaction distances for all prey types were similar during day and night. Despite longer reaction distances during the day, galaxias still managed to consume similar total numbers of prey items during the day and night, except that *Deleatidium* consumption declined at night. Galaxias consumed more fast-swimming *Nesameletus* than slow-swimming *Deleatidium*. This pattern of consumption, and the short movements punctuated by frequent stops observed while galaxias were foraging at night, suggest that *G. vulgaris* is using mechanical cues to detect and capture prey (Montgomery and Milton 1993; Halstead 1994). Furthermore, their lack of reaction when approaching mayflies in the benthos until contacting or disturbing them is also consistent with the use of mechanical cues to detect prey. It seems that visual cues increase reaction distance for common river galaxias during the day but the fish relies primarily on its lateral line and mechanosensory system to locate prey, particularly at night when vision is ineffective. Thus, prey producing larger mechanical stimuli, such as *Nesameletus*, will be more vulnerable to galaxias predation.

Galaxias movements at night often disturbed mayflies from the substratum. McIntosh and Townsend (1994) concluded that these disturbances were likely to be responsible for nocturnal increases in *Nesameletus* drift in streams with galaxias. It is possible that by moving around at night galaxias are actively disturbing prey from the benthos, causing them to enter the water column where we have shown they are most often consumed.

Trout behaved very differently. Their reaction distances and consumption of prey were greater during the day. They detected most prey from the water column and their reaction distances were most affected by prey size. This description of brown trout foraging agrees with the established stereotype of trout as size-selective, drift feeders that rely primarily on visual cues (Ware 1972; Metz 1974; Allan 1978, 1981; Walsh et al. 1988; Angradi and Griffith 1990). It is important to note that brown trout in our experiments captured as many prey items as galaxias during the night. This indicates they can use cues other than vision to detect and capture prey as efficiently as galaxias. Other accounts of trout foraging also show they are capable of capturing large numbers of prey during the night (Jenkins 1969; Elliott 1970).

In our field investigation of stomach contents we observed differences in diet composition and in the number of aquatic and terrestrial prey consumed, but no consistent trends in the diel pattern of consumption of aquatic prey for either fish species. Furthermore, the analysis of aquatic prey biomass with analysis of covariance (ANCOVA) revealed no significant differences between the consumption by galaxias and trout. The significant covariate, fish weight, in the ANCOVA indicates that the differences in prey consumption shown in Fig. 5*b* were caused by the size differences between trout and galaxias. Thus, per unit of fish biomass, trout and galaxias are likely to consume similar amounts of aquatic prey.

These patterns of consumption from real streams will have been influenced by the predator avoidance abilities of the prey, which we specifically tried to minimize in the laboratory. When the results of our laboratory experiments are combined with a knowledge of the predator avoidance behaviours of the prey in streams with trout and galaxias (McIntosh 1994; McIntosh and Townsend 1994) the consumption patterns observed in the field can be interpreted in terms of the predation risk presented by trout and galaxias.

Our experiments indicate that the timing of the predation threat presented by trout is different from that presented by galaxias. Mayflies were equally vulnerable to common river galaxias during the day and night but were more vulnerable to brown trout during the day. This pattern was shown in our field survey of stomach contents for common river galaxias but not brown trout. Differences in the availability of prey in the field are likely to be responsible for this apparent contradiction. A large amount of evidence now suggests that many aquatic prey (particularly mayflies) show nocturnal drift periodicities whenever visually feeding fish are present (Brittain and Eikeland 1988; Flecker 1992; Douglas et al. 1994; McIntosh and Townsend 1994). We also have evidence that mayflies in the trout streams surveyed are more active at night (McIntosh and Townsend 1994). The diel patterns in gut contents from the trout streams are therefore likely to be confounded by increases in prey availability at night. We used prey from a fishless stream in our laboratory experiments to avoid the possibility that our estimates of predation risk would be biased by differences in prey behaviour. Thus, the laboratory experiments are likely to be a more reliable indicator of the timing of predation risk and the patterns in the field gut contents of trout are consistent with our contention that brown trout present a higher predation risk during the day.

Cadwallader (1975*a*) observed that *G. vulgaris* did not attempt to feed during the day, even when offered prey, and other studies have indicated that common river galaxias feed more during the night and have more in their stomachs at night (Glova and Sagar 1989*b*, 1993; Cadwallader 1975*b*, 1975*c*; Glova et al. 1992). In our experiments, galaxias consumed just as many prey items during the day as at night. The artificial nature of the stream channels that optimized feeding would have increased prey consumption, but is unlikely to be responsible for this different diel pattern because flow conditions and prey availability in the channels were exactly the same during the day and night. Instead, these differences are more likely to be explained by the effect of trout on prey behaviour. All of the previous examinations used common river galaxias from streams where brown trout, or other drift-feeding salmonids, were also present. Thus, the nocturnal feeding patterns of common river galaxias in these streams may be explained by the increase in prey availability during the night as described previously. A possible alternative explanation is that competitive interactions between salmonids and common river galaxias restrict galaxias to nocturnal foraging in situations where both species occur (Edge et al. 1993; McIntosh et al. 1994).

Our experiments showed large differences in the foraging strategies of the two fish predators. However, these do not necessarily correspond to differences in the spatial distribution of predation risk. Both trout and galaxias detected most *Nesameletus* and *Deleatidium* while they were in the water column. The aquatic diet composition of brown trout from the streams examined was dominated by mayflies but the diet of common river galaxias was more evenly spread among a number of taxa. This could indicate that trout were feeding more on drifting items as mayflies are known to have high drift rates (Brittain and

Eikeland 1988). However, diet composition in the field is also likely to be confounded by differences in prey availability and prey behaviour. Other studies of foraging by brown trout and common river galaxias suggest that both catch the majority of their prey from the water column (Cadwallader 1975*c*; Glova and Sagar 1989*b*). Further experiments considering predation upon different prey types, or prey showing different behaviours, are needed to determine if certain prey types (or behaviours) are more or less vulnerable to predation by brown trout.

One important question that might now be asked is: has the total consumption of prey changed in streams where trout have been introduced? Brown trout consumed more than common river galaxias per individual in the laboratory and the field. However, when the obvious differences in fish weight were taken into account in the ANCOVA there was no significant difference in the consumption of aquatic prey in the field by trout and common river galaxias. Densities of brown trout are lower than densities of common river galaxias in tributaries of the Taieri River (0.3 ± 0.03 and 1.03 ± 0.09 $m^{-2}$, respectively) but trout weigh more than galaxias (13.87 ± 0.99 and 3.72 ± 0.08 g, respectively) and streams with trout and streams with galaxias have similar fish biomasses (4.05 ± 0.49 and 3.42 ± 0.37 $g \cdot m^{-2}$, respectively) per unit area (C.R. Townsend, University of Otago, Dunedin, New Zealand, unpublished results). Thus, the introduction of trout has probably not resulted in a large change in total prey consumption.

Our consideration of the impact of the introduction of brown trout on the fish predation regime of streams in the Taieri River System has revealed that although the exotic predator behaves very differently from the native predator, changes in the predation regime have been subtle. The introduction of brown trout has changed the timing of the predation threat and the strategy used to capture prey but may not have altered overall levels of predation. However, these alterations in the predation regime have dramatically altered prey behaviour. *Nesameletus* from streams containing brown trout have apparently evolved fixed nocturnal periodicities; by hiding during the day they reduce the high risk of trout predation at this time (McIntosh and Townsend 1994). In contrast, *Nesameletus* from streams without trout use a flexible behaviour strategy and avoid galaxias when they are present. Changes in the predation regime are also likely to have important consequences for lower trophic levels, through trophic cascades. Recent evidence indicates that *Deleatidium* graze less when trout are present, resulting in increases in algal standing crop (McIntosh 1994). This means that the subtle, but important, changes in the predation regime caused by the introduction of brown trout reported here are likely to have far-reaching effects in many New Zealand streams.

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## Appendix

Relative abundance (%) of recently eaten invertebrate taxa in the stomachs of common river galaxias and brown trout during the day and the night in sites in the Taieri River system.

| | | Galaxias | | Trout | |
|---|---|---|---|---|---|
| | Classification | Day ($N$ = 40) | Night ($N$ = 42) | Day ($N$ = 39) | Night ($N$ = 46) |
| Aquatic organisms | | | | | |
| Ostracoda | O | 1.5 | — | — | — |
| Gastropoda | | | | | |
| *Potamopyrgus antipodarum* | O | 2.9 | 6.0 | 0.3 | — |
| Megaloptera | | | | | |
| *Archichauliodes diversus* | P | — | — | — | 0.7 |
| Ephemeroptera | | | | | |
| *Coloburiscus humeralis* | M | 1.5 | — | 2.2 | 1.3 |
| *Deleatidium* spp. | M | 26.1 | 19.4 | 57.0 | 63.5 |
| *Nesameletus* spp. | M | — | 1.5 | 2.2 | 1.8 |
| Plecoptera | | | | | |
| *Stenoperla prasina* | P | 1.5 | — | — | — |
| *Zelandoperla* spp. | O | — | — | — | 0.2 |
| *Zelandobius* spp. | O | — | 1.5 | — | — |
| Trichoptera | | | | | |
| *Aoteapsyche* spp. | C | 1.5 | 1.5 | 1.5 | 1.3 |
| *Bareoptera* spp. | C | — | — | 1.1 | 1.6 |
| *Hudsonema amabilis* | C | 5.8 | 16.4 | 2.6 | 17.7 |
| *Hydrobiosis* spp. | P | 4.4 | 3.0 | 2.2 | 3.1 |
| *Olinga feredayi* | C | — | — | 2.2 | 0.2 |
| *Oxyethira albiceps* | C | 2.9 | 1.5 | 1.8 | 0.7 |
| *Polyplectropus* spp. | P | 2.9 | 6.0 | 0.4 | 0.2 |
| *Psilochorema* spp. | P | 5.8 | 3.0 | 1.1 | 0.9 |
| *Pycnocentrodes* spp. | C | 21.7 | 3.0 | 17.6 | 6.9 |
| *Pycnocentria erecta* | C | — | — | — | 1.3 |
| Unidentified | C | 1.5 | 3.0 | — | 0.2 |
| Coleoptera | O | 2.9 | 3.0 | — | 0.2 |
| Diptera (larvae) | | | | | |
| *Austrosimulium* spp. | O | 1.5 | 3.0 | 1.5 | 1.1 |
| Chironomidae | O | 1.5 | 3.0 | 6.3 | 6.4 |
| Tipulidae | O | 5.8 | 10.5 | — | — |
| Decapoda | O | 1.5 | — | — | — |
| Amphipoda | O | 3.0 | 9.0 | 0.3 | 3.3 |
| Terrestrial organisms | | | | | |
| Oligochaeta | T | 0.9 | — | — | — |
| Araneae | T | — | 2.3 | — | — |
| Orthoptera | T | — | 1.5 | — | — |
| Diptera (adults) | T | 71.2 | 48.0 | 6.1 | 2.8 |

**Note**: Classifications used to group prey items are as follows: M, mayflies; C, nonpredatory caddisflies; P, invertebrate predators; O, others; T, terrestrials.

# Effects of habitat on settlement, growth, and postsettlement survival of Atlantic cod (*Gadus morhua*)

**M. Tupper and R.G. Boutilier**

**Abstract**: Settlement and growth of age 0+ cod were monitored using snorkel and self-contained underwater breathing apparatus (SCUBA) in four distinct habitat types (sand, seagrass, cobble, and rock reef) in St. Margaret's Bay, Nova Scotia. Newly settled cod were marked with acrylic dye, allowing repeated visual length estimates of individual fish. Settlement of cod did not differ between habitat types, but postsettlement survival and subsequent juvenile densities were higher in more structurally complex habitats. These differences appear to be due to increased shelter availability and decreased predator efficiency in structurally complex habitats. Growth rate was highest in seagrass beds, while the efficiency of cod predators was lowest and cod survival was highest on rocky reefs and cobble bottoms. Thus, trade-offs occur between energy gain and predation risk. In St. Margaret's Bay, the population structure of Atlantic cod may be less influenced by patterns of larval supply than by postsettlement processes such as habitat-specific growth and mortality.

**Résumé** : Nous avons surveillé la colonisation et la croissance de morues d'âge 0+, en plongée autonome et avec masque et tube, dans quatre types différents d'habitats (sable, herbier, galets, récifs rocheux) dans la baie St. Margaret's, en Nouvelle-Écosse. Les morues nouvellement installées étaient marquées avec une peinture acrylique, ce qui a permis de mesurer visuellement de façon répétée la longueur des poissons pris individuellement. Le mode de colonisation ne différait pas d'un habitat à l'autre, mais la survie après installation et la densité des juvéniles par la suite étaient plus élevées dans les habitats structurellement plus complexes. Ces différences semblent dues à une plus grande disponibilité des abris et à une efficacité plus faible des prédateurs dans les habitats structurellement complexes. Le taux de croissance était au plus haut dans les herbiers, tandis que l'efficacité des prédateurs de la morue était au plus bas et la survie des poissons au plus haut sur les récifs rocheux et les fonds de galets. Il s'établit donc des compromis entre le gain d'énergie et le risque de prédation. Dans la baie St. Margaret's, la structure de la population de morue franche semble être moins tributaire du régime d'apport de larves que des processus post-colonisation comme la croissance et la mortalité spécifiques à un habitat.

[Traduit par la Rédaction]

## Introduction

Variability in the survival of early life-history stages of fish is proposed to be a major factor limiting the size of adult populations. While studies of recruitment variability have largely focused on survival of planktonic larvae (Doherty 1982, 1983; Victor 1983, 1986; Doherty and Fowler 1994; Williams et al. 1994), recent evidence has pointed to early juvenile survivorship as an important contributor to recruitment variation (Hunt von Herbing and Hunte 1991; Jones 1991; Malloy and Targett 1991; de Lafontaine et al. 1992; Tupper and Hunte 1994; Tupper and Boutilier 1995). However, little is known about the processes regulating settlement and survival of age 0+ (first-year juvenile) fish in northern marine ecosystems.

Spatial variation in demersal fish recruitment is often perceived as habitat selection, particularly when recruitment to a specific habitat is disproportionately high relative to the abundance of that habitat (Carr 1991). While some fish species may actively select specific microhabitats at settlement (Sale et al. 1984), recruitment variation among habitats may result from a number of factors: (*i*) among-habitat variation in the availability of presettlement larvae, (*ii*) differential availability of shelter from predation, i.e., habitat complexity (Shulman 1985; Behrents 1987; Hixon and Beets 1989; Carr 1994), or (*iii*) agonistic interactions with conspecifics or competitors (Sweatman 1985, 1988). Predation efficiency of piscivorous predators has been shown to decrease with increasing habitat complexity (Mattila 1992). Thus, habitats characterized by high substrate complexity

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should favour postsettlement survival through decreased predation pressure.

The high mortality rates typically associated with postsettlement juveniles are thought to be primarily due to predation (Houde 1987). Many piscivorous fishes select smaller size-classes (Juanes 1994). In addition, overwinter survival of young-of-the-year fish in northern climates is generally higher in larger individuals (Walsh 1987; Henderson et al. 1988). Thus, rapid growth of newly settled fish may confer a selective advantage both physiologically and in terms of reduced predation risk. In general, we should expect higher survival rates in those habitats providing the greatest potential for growth. However, conflicts in habitat selection may occur if those habitats providing maximum growth are not also those that provide the maximum amount of shelter from predation (Sogard 1992). In such cases, high predation risk may force newly settled fish into habitats that do not optimize growth or, alternatively, insufficient food abundance may force newly settled fish into a habitat where the risk of predation is significantly higher.

Studies of these effects of habitat on the demography of juvenile fishes have focused mainly on reef-associated species (e.g., Sale et al. 1984; Carr 1991; Connell and Jones 1991; Levin 1991, 1993). Indeed, much of what is known about the recruitment dynamics of demersal marine fishes stems from studies of coral reef fishes. In this paper, we applied a sampling approach typical of reef fish studies to the juvenile demographics of the Atlantic cod (*Gadus morhua*), a key commercial species in the temperate North Atlantic region. We compared settlement, growth, and postsettlement survival of individually tagged age 0+ Atlantic cod in four distinct habitat types. In addition, we measured the density and capture success of predators on juvenile cod in each habitat. The goal of the study was to answer the following questions. (*i*) Do settlement, growth, and survival of age 0+ cod vary among habitat types? (*ii*) Do predator densities and capture success rates influence survival of age 0+ cod in each habitat? (*iii*) Is the among-habitat pattern of settlement preserved in the juvenile population or modified by mortality and (or) emigration?

## Methods

### Study locations

The study was conducted at three locations along the shore of St. Margaret's Bay, Nova Scotia. The nearshore bottom of St. Margaret's Bay can be divided into four broad habitat types: rocky reef (characterized by bedrock strewn with large granitic boulders), cobble bottom, seagrass (*Zostera marina*) beds, and sand bottom. Macroalgae were scarce at these sites, owing to herbivorous grazing by the sea urchin *Strongylocentrotus drobachiensis*. Rocky reefs were typically covered by crustose algae and (or) a short turf of filamentous algae, neither of which would provide suitable shelter for juvenile cod.

At each location, four 15-m transect lines were laid in each of the four habitat types (i.e., 16 transects/location). All transects were within 100 m of shore, at a depth of 1.5–2 m (mean low tide). The substrate rugosity (a measure of habitat complexity, see Chandler et al. 1985) of each transect site was estimated by fitting a fine-link brass chain to the bottom contours along the transect line. The total distance covered by the chain was then divided by 15 m (the horizontal distance covered by the transect line), producing an index of substrate rugosity. It is important to note that substrate rugosity is simply a measure of the actual surface area of bottom structure available to an organism and does not account for other possible shelter sites, such as that provided by seagrasses. However, even in dense seagrass beds, newly settled cod in this study were not closely associated with vegetation, but sought refuge within the interstices of rocks, shells, and other hard bottom (M. Tupper, personal observations).

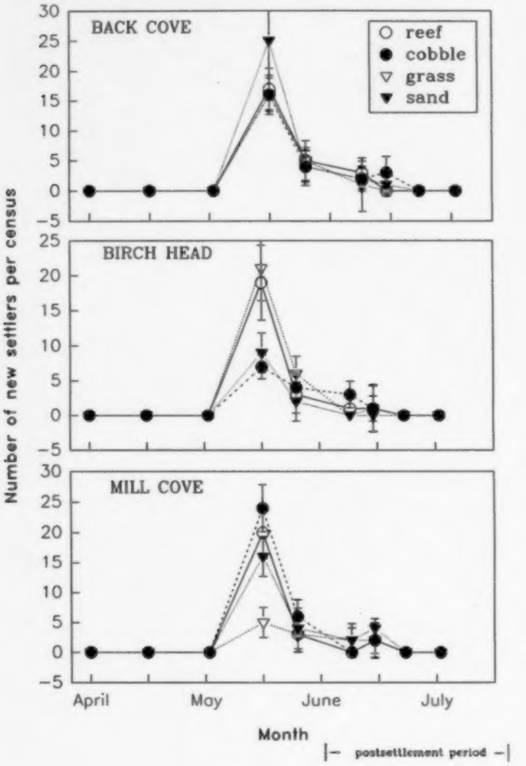
### Census techniques

Visual censuses of juvenile cod abundance were conducted at roughly 10-day intervals from May 1 to July 1, 1992, by snorkelling slowly along the transect lines and counting all cod within 1 m on either side of the transect line (30 $m^2$ planar surface area). Settlement was measured as the mean number of newly settled cod on the four transect lines at each transect site. Newly settled cod were recognizable as being <60 mm in total length and having a barred pigmentation pattern and a silvery underbelly. This pattern slowly fades until the adult pigmentation is assumed at 1–2 weeks postsettlement (M. Tupper, personal observations). Newly settled cod were anaesthetized in situ using a 10% solution of Quinaldine in seawater. The total length of each animal was measured to the nearest millimetre with a 100-mm plastic ruler, and the animal was then marked with injections of acrylic paint, using a specific pattern of dots to identify individuals. This allowed marked individuals to be recognized as prior residents in subsequent censuses, and allowed growth, mortality, and emigration to be quantified at each site. The length of each fish was estimated visually at each census using a clear plastic ruler fastened to the end of a 60 cm length of wooden dowel. The procedure was facilitated by the behaviour of young-of-the-year cod, which tend to flatten themselves against the substrate and remain immobile when approached. This technique was accurate to ±3.9 mm for cod under 100 mm in total length (Tupper and Boutilier 1995). Individual growth rates of tagged fish were calculated as the increase in total length divided by the number of days since the previous study in July, divided by the total number of settlers tagged on that transect. Individual growth rates of tagged fish were calculated as the increase in total length divided by the number of days since the previous measurement. Continuous water temperature records were not available for the specific study sites, but were available from an aquaculture site at the head of St. Margaret's Bay approximately 2 km from the study sites. Water temperatures on each transect were recorded immediately following a census with a mercury thermometer.

### Predation

Observations of predation by other fishes on juvenile cod were made in situ at roughly 10-day intervals from mid-May to early July, 1992, at each transect site. The observer floated motionless above the site while recording the species and number of predators, the number of attempted strikes

**Fig. 1.** Temporal variation in settlement of age 0+ cod to different habitat types (rocky reef, cobble bottom, seagrass beds, and sandy bottom) at three sites in St. Margaret's Bay, Nova Scotia. Error bars represent ±1 standard deviation.



(lunges at a juvenile cod), and the number of successful strikes (capture and ingestion of a juvenile cod) over a 1-h observation period. An index of capture success was then calculated by dividing the number of successful strikes by the total number of strikes, for each of the four habitat types. Mean capture success in each habitat was calculated as the average capture success over the six observation periods. Predation was initially observed at dawn, in the afternoon, at dusk, and at night. However, the time of observation appeared to have no effect on the number or species of predators on the transects, and observations were therefore continued during the afternoon only.

### Data analysis

Settlement rates, population densities, and mean growth rates were compared among sites and habitats using two-way analysis of variance (ANOVA) (Sokal and Rohlf 1981). Sites and habitats were considered fixed treatment effects, while censuses were considered random effects and a repeated measure. All numeric data were log $(x + 1)$ transformed prior to analysis to ensure homoscedasticity (Bartlett's test for homogeneity of variance (Wilkinson 1990)). Predator densities and capture success rates were also compared among locations and habitats using two-way analysis of variance. To determine where significant differences among locations or habitats occurred, post hoc analyses were conducted using Tukey's honestly significant difference test (HSD) (Wilkinson 1990). Linear regression analyses were used to determine the effects of substrate rugosity on settlement, postsettlement survival, predator success, and growth rate.

## Results

### Variation in environmental variables

Water temperature throughout the study ranged from 4°C in early May to 9°C in early July. On any sampling day throughout the study, there was less than 1°C difference in temperature between locations and between transect sites at a location. The general uniformity of temperature across the lower reaches of St. Margaret's Bay is most likely due to regular tidal flushing. It was therefore assumed that differences in growth between habitats or locations were not attributable to spatial variations in temperature.

Mean substrate rugosity (measured for each habitat type as the average of all transects in that habitat type) differed between the four habitat types (ANOVA, $F = 9.05$, $p < 0.001$). The mean values (1.38 for sand, 2.04 for grass, 3.37 for cobble, and 3.76 for reef) differed in all pairwise comparisons (Tukey's HSD, $p < 0.05$ for all comparisons). We are therefore confident that the four habitat types chosen represent real differences in terms of the amount of shelter available to newly settled cod.

### Among-habitat variation in settlement and population density

Comparisons between transects revealed no differences in the timing or strength of settlement among sites or habitats. No juvenile cod were observed on any of the transect sites upon initiation of the monitoring program in early April. Juvenile cod settled mostly in one pulse during the 2nd and 3rd weeks of May (Fig. 1). The timing of settlement was generally similar across locations and across habitat types within a location, i.e., at scales of several kilometres or several metres. No significant differences ($p > 0.05$) in settlement were found between habitat types or between sites (Table 1), although a significant location-by-habitat interaction was detected (Table 1). The settlement of juvenile cod and the substrate rugosity of the transect sites were not correlated ($r^2 = 0.2$, $p > 0.05$).

The population density of age 0+ cod rose rapidly at all transect sites following the pulse of settlement in mid-May, then began to decrease in late May or early June (Fig. 2). The decline in population density was attributed to postsettlement mortality rather than emigration, because behavioural studies of age 0+ cod in St. Margaret's Bay revealed them to be strongly site attached for the first 2–3 months of demersal life (Tupper and Boutilier 1995). In addition, marked individuals were not found in monthly monitoring of the areas surrounding the transect sites. The decline in density was much greater in sand and seagrass habitats than in the structurally more complex cobble and reef habitats (Fig. 2). At all three locations, population densities of age 0+ cod on sand bottom reached 0 by late

June. Substrate rugosity accounted for 75% of the variance in postsettlement survival ($p < 0.0001$; Fig. 3). In summary, despite a uniform pattern of settlement among habitats and locations, survival varied significantly between habitats and was positvely correlated with the complexity of the habitat. These results suggest that demersal young-of-the-year cod in St. Margaret's Bay are limited by the availability of shelter sites, and that suitable nursery habitats for age 0+ cod should provide an abundance of refugia. The fact that population density in a given habitat did not differ between locations presumably reflects a combination of the spatially uniform pattern of cod settlement in St. Margaret's Bay with a similarity between locations in the postsettlement processes controlling among-habitat variations in population density.

### Predation on juvenile cod

Three species of Cottidae (*Hemitripterus americanus*, *Myoxocephalus octodecemspinosus*, and *Myoxocephalus aeneus*) were observed preying on age 0+ cod in St. Margaret's Bay. Because these three species have very similar behaviours, they were pooled as cottid predators. Predator densities did not vary among habitat types but did vary among locations (two-way ANOVA, Table 1). A total of 96 predator–prey interactions (attempted captures) were observed during the course of the study. The number of attempted captures did not vary significantly between habitat types or locations (Table 1). Capture success varied among habitats but not among sites (two-way ANOVA; Table 1). Predators were most successful on open sand bottoms and least successful in reef and cobble habitats. Capture success on a given transect was not related to predator density ($r^2 = 0.09$, $p > 0.3$) but was inversely related to substrate rugosity ($r^2 = 0.74$, $p < 0.001$; Fig. 4). Because the among-habitat pattern of capture success is inversely related to the survival of age 0+ cod, it appears that predation is a major factor influencing variation among habitats in recruitment of young-of-the-year cod.

### Among-habitat variation in growth

There was no difference in mean size at settlement between habitat types or locations (ANOVA, $F = 1.43$, $p > 0.05$). Significant differences in mean growth rate were observed among habitat types but not among locations (Table 1). There was no significant location-by-habitat interaction ($F = 0.35$, $p > 0.9$). At all three locations, the growth rate of juvenile cod was highest in seagrass habitats (range 0.59–0.67 mm·day$^{-1}$) and lowest on sand bottoms (range 0.02–0.06 mm·day$^{-1}$). Growth rates did not significantly differ between reef (range 0.38–0.45 mm·day$^{-1}$) and cobble (range 0.35–0.41 mm·day$^{-1}$) habitats at any of the three locations (Table 1).

## Discussion

### Settlement of young-of-the-year cod

In this study, juvenile cod did not demonstrate any preference for habitat type at settlement (Table 1). In contrast to patterns of settlement, survival of juvenile cod varied greatly between habitat types (Table 1). The processes regulating among-habitat variation in juvenile cod density

**Table 1.** Variation in settlement, survival, and growth of age 0+ cod, and predation on age 0+ cod by cottid predators.

| Location | Substrate | Settlement (no. settlers/ transect) | | % survival | | Predator density ((no. cottids/ transect)/h) | | Attempted strikes ((no. strikes/ transect)/h) | | Captures ((no./ transect)/h) | | % capture success (no. captures/ no. attempts) | | Growth rate (mm/day) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Back Cove | Reef | 25.5 | 7.8 | 43.5 | 4.4 | 1.1 | 0.34 | 1.5 | 1.0 | 0.2* | 0.5 | 13.2* | 3.2 | 0.37 | 0.03 |
| | Cobble | 25.3 | 6.2 | 36.3 | 8.4 | 0.7* | 0.38 | 2.5 | 1.0 | 0.6 | 0.5 | 23.5* | 1.7 | 0.34 | 0.03 |
| | Grass | 22.0 | 7.2 | 20.9* | 3.5 | 0.9 | 0.24 | 2.5 | 2.1 | 1.0 | 0.7 | 39.7* | 5.0 | 0.59* | 0.03 |
| | Sand | 32.0 | 7.3 | 0.0* | 0.0 | 1.3 | 0.27 | 2.0 | 0.8 | 1.2* | 0.8 | 60.1* | 10.3 | 0.02* | 0.02 |
| Birch Head | Reef | 24.5 | 6.3 | 39.0 | 2.0 | 1.0 | 0.40 | 1.8 | 1.3 | 0.3* | 0.5 | 19.2* | 1.7 | 0.38 | 0.02 |
| | Cobble | 15.5* | 6.5 | 28.5* | 1.7 | 1.5 | 0.21 | 2.0 | 1.8 | 0.7 | 0.6 | 32.8* | 9.0 | 0.40 | 0.02 |
| | Grass | 28.3 | 7.9 | 15.0* | 2.4 | 1.8* | 0.42 | 1.5 | 1.0 | 0.8 | 0.5 | 50.0* | 0.0 | 0.63* | 0.05 |
| | Sand | 11.8* | 5.4 | 0.0* | 0.0 | 1.2 | 0.35 | 2.5 | 1.0 | 1.8* | 1.0 | 73.0* | 2.4 | 0.06* | 0.01 |
| Mill Cove | Reef | 25.5 | 4.8 | 34.8 | 1.1 | 0.9 | 0.24 | 2.0 | 0.0 | 0.4* | 0.5 | 21.3* | 3.0 | 0.45 | 0.03 |
| | Cobble | 32.0 | 8.2 | 31.3 | 1.6 | 1.1 | 0.32 | 2.0 | 1.9 | 0.7 | 0.7 | 33.7* | 6.1 | 0.39 | 0.04 |
| | Grass | 12.8* | 5.9 | 13.8* | 2.5 | 1.2 | 0.45 | 1.5 | 1.0 | 0.9 | 0.8 | 59.2* | 5.5 | 0.67* | 0.02 |
| | Sand | 26.0 | 6.8 | 0.0* | 0.0 | 0.7* | 0.59 | 2.5 | 1.8 | 1.9* | 0.7 | 76.8* | 2.4 | 0.03* | 0.01 |

**Note:** Capture success is the percentage of attempted strikes that are successful.
*Significantly different from other habitats at that location ($p < 0.05$).

**Fig. 2.** Temporal variation in population density (no. of individuals per 15-m transect) of age 0+ cod in four habitat types (rocky reef, cobble bottom, seagrass beds, and sandy bottom) at each of three sites in St. Margaret's Bay, Nova Scotia. Error bars represent ±1 standard deviation.

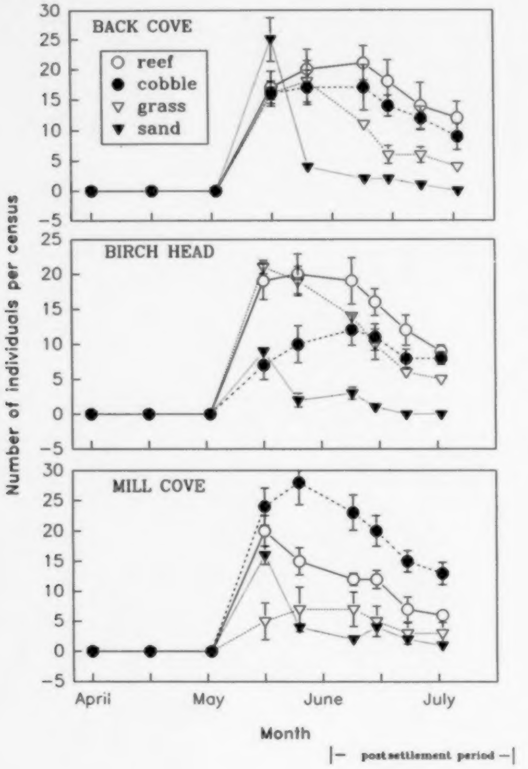
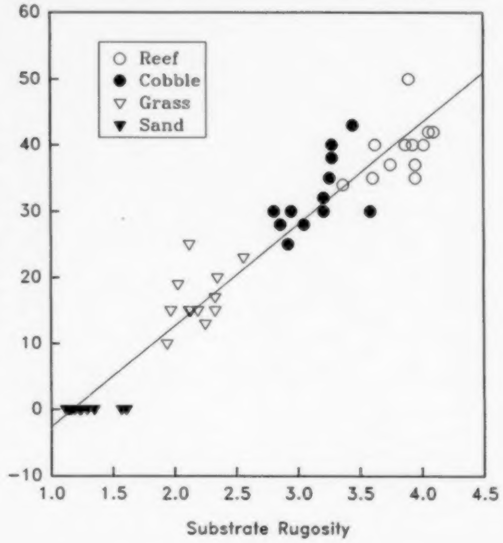


**Fig. 3.** Effect of substrate rugosity of a transect on postsettlement survival of age 0+ cod. Survival is calculated as the percentage of all fish settling onto a given transect that survived until the end of the experiment.



must therefore occur postsettlement, with differential survival rather than differential settlement leading to the observed patterns of density. In contrast, Sale et al. (1984) clearly demonstrated that several species of coral reef fish settle preferentially to specific microhabitats, and suggested that spatial variation in microhabitat structure may result in spatial variation in settlement and subsequent recruitment.

The relative importance of settlement and postsettlement processes in determining fish–habitat associations is unknown for most species (Jones 1991). Habitat type may influence postsettlement processes to either reinforce or alter spatial patterns established at settlement. For example, the damselfish *Pomacentrus amboinensis* settles preferentially in deeper lagoonal waters, where its subsequent growth rate and survival are higher than in shallow water (Jones 1986). In contrast, the blennoid fish *Forsterygion varium* settles uniformly among habitat types differing in structural complexity, but exhibits extreme differences in subsequent survival and density, which are much higher in more complex habitats (Connell and Jones 1991). This pattern is identical to that exhibited by juvenile cod in this study. As with *F. varium*, among-habitat variation in the survival of juvenile cod radically altered patterns of settlement.

### Influence of habitat structure and predation on juvenile demography

The existence of a positive correlation between survival of juvenile fish and habitat complexity is considered evidence for space limitation, i.e., intraspecific competition for limited shelter sites (e.g., Shulman 1985). In this study, the efficiency of cottid predators decreased with increasing habitat complexity, and similar results were observed for predation on amphipods by yellow perch, *Perca flavescens*, and ruffe, *Gymnocephalus cernuus* (Mattila 1992). Gotcietas and Brown (1993) found that young-of-the-year cod kept in aquaria selected more complex substrates in the presence of a predator. Thus, the relationship between habitat complexity and survival need not be explained solely by competition for living space, but can also be attributed to differential predation among habitat types. Thus, a very high predator density in more complex habitats could still lead to higher predation mortality in those habitats. However, in St. Margaret's Bay, predator density did not vary significantly between habitats. As a result, survival remained positively correlated with habitat complexity (Fig. 3).

Mattila (1992) suggests that predation efficiency is not automatically reduced by a high level of habitat complexity per se; the prey must be able to utilize that complexity as an effective shelter. In this study, seagrass beds could conceivably provide the greatest complexity of the four habitats, if the vertical area of each blade of grass were measured. However, young-of-the-year cod did not utilize seagrass blades as shelter: they preferred rocks, empty scallop shells, and other debris found within the seagrass beds. Considering the rapid postsettlement growth rates of young-of-the-year cod, it seems probable that they essentially outgrow seagrass blades as a suitable refuge soon after settlement. Connell and Jones (1991) hypothesized that the lack of habitat-specific settlement in *F. varium* may be due to settlement-sized fish finding adequate shelter in a wide range of habitats, while habitat-specific mortality may arise when settled fish outgrow suitable shelter in habitats of lower complexity. This hypothesis fits our findings for young-of-the-year cod equally well. If cod, at their smallest sizes, can find shelter in all habitats more or less equally, differences in settlement strength between habitats would not be expected. Patterns of distribution in juvenile cod populations would then be determined by habitat-specific predation pressure. Lough et al. (1989) offered a similar hypothesis to explain the demography of young-of-the-year cod and haddock on Georges Bank. They observed that pelagic juvenile gadids were widespread over the bank in June, but that in July, demersal juveniles were found mainly on pebble–gravel beds and were "poorly represented" on other, less complex sedimentary substrates. They also suggested that in addition to providing more shelter sites than the other substrates, the pebble–gravel deposits most closely matched the mottled colouring of demersal juvenile cod, making the small fish very difficult to see, and therefore reducing their predation risk. From these results, Lough et al. (1989) concluded that the availability of a complex habitat with reduced predation pressure may be critical to the recruitment success of cod and haddock on Georges Bank.

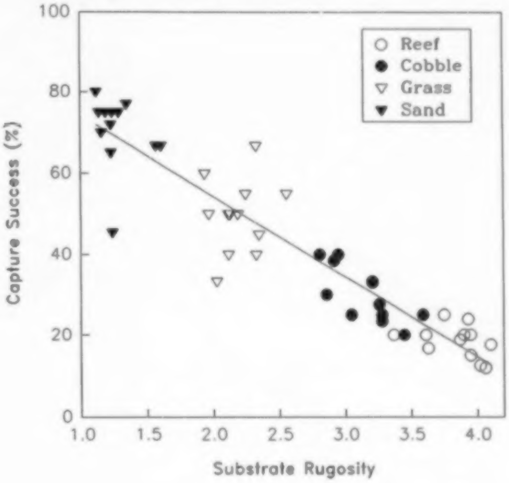
### Influence of habitat structure on growth

The growth rates of young-of-the-year cod were somewhat lower than previously published values for fish of this size. Bolz and Lough (1988) reported growth rates of 0.71 mm·day$^{-1}$ for cod of 31 mm standard length, to 1.09 mm·day$^{-1}$ for cod of 67 mm standard length. In the present study, mean growth rate over all habitats was approximately 0.4 mm·day$^{-1}$. However, Bolz and Lough studied populations on Georges Bank during the summer and fall months; these fish were growing at higher temperatures than the spring cohort of young-of-the-year cod from St. Margaret's Bay.

Connell and Jones (1991) suggested that habitat complexity may influence the growth and survival of juvenile fish through increased prey density and prey diversity. The growth rate of young-of-the-year cod in this study differed between habitats, but was not related to substrate rugosity per se. However, as discussed in the previous section, the shelter provided by vegetation was not accounted for in this measure of complexity. For small prey items, seagrass beds undoubtedly offer the greatest habitat complexity. For example, Sogard (1992) reported that the presence of seagrass beds increased copepod densities by 49% and amphipod densities by 64%. Thus, if habitat complexity is measured with regard to prey size rather than predator size, then prey densities and predator growth rates should increase in more complex habitats.

In this study, young-of-the-year cod experienced elevated growth rates in seagrass beds (presumably because of the higher prey densities within this habitat), but experienced significantly reduced predation risk in rocky reef and cobble habitats. It remains unclear which strategy is the more advantageous for young-of-the-year cod. Several researchers (Werner and Hall 1988; Connell and Jones 1991; Sogard 1992) have suggested the possibility of trade-offs in habitat utilization by fish. Present ecological theory tells us that fish should select for the habitat that maximizes energy gain (growth), while minimizing the risk of mortality. Cod in St. Margaret's Bay appear to lose their site fidelity by their first winter, at which point they disperse from shallow nearshore habitats, presumably joining the older juvenile population in deeper, warmer water (Tupper and Boutilier 1995). The greatest numbers of fish undergoing this migration will be from complex hard-bottom habitats (reef and cobble), while the largest individuals will be from seagrass habitats. Overwinter survival of several temperate fishes has been demonstrated to be size dependent, with the largest individuals surviving best (Henderson et al. 1988). Thus one can foresee a scenario in which one habitat supplies the population with a greater number of smaller recruits, each with a somewhat lesser chance of survival, while another habitat supplies fewer, larger recruits, each with a relatively high chance of survival. Further research is needed to establish the relative importance of these differing strategies to the population dynamics of Atlantic cod.

**Fig. 4.** The effect of substrate rugosity on capture success of cottid predators on age 0+ cod. Capture success is calculated as the number of successful captures divided by the number of attempted captures.



### Conclusions

Over the past decade, studies of demersal marine fish recruitment have generally concluded that recruitment variation results mainly from stochastic larval survival (Doherty 1982, 1983; Victor 1983, 1986; Houde 1987; Miller et al. 1988; Beyer 1989; Pepin and Myers 1991; Milicich et al. 1992; Doherty and Fowler 1994). Recently a more pluralistic view has been suggested, in which both pre- and post-settlement events are considered important in regulating adult population size (Forrester 1990; Jones 1990; Jones 1991; Hunt von Herbing and Hunte 1991; Tupper and

Hunte 1994; Tupper and Boutilier 1995). The relative importance or pre- and post-settlement events in determining recruitment strength will vary with location, time, and habitat (Jones 1990; Connell and Jones 1991). While almost all research in this field has been conducted on coral reef fishes, similar mechanisms seem to apply to young-of-the-year Atlantic cod. In this study, spatial patterns of settlement of cod were completely altered by habitat-specific postsettlement mortality. In addition, growth rates of young-of-the-year cod were influenced by habitat type, which may in turn influence overwinter survival. However, in years or locations in which larval survival and settlement were very low, the importance of these postsettlement processes would likely decrease, and the abundance of young-of-the-year cod would be regulated mainly by larval input. Longer term studies are needed to assess the importance of pre- and post-settlement events over several years (Forrester 1990; Jones 1990).

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# Size-related changes in diel distribution of young grayling (*Thymallus thymallus*)

**P. Sempeski and P. Gaudin**

**Abstract**: An approach based on direct observation from the riverbank allowed a precise description of the size-dependent distribution patterns of young grayling. The smallest larvae (15–20 mm) were mainly observed in the upper layers of the water column and very close to the riverbank. With increasing age and size (20–35 mm), old larvae and young parr began to hold positions closer to the bottom and to the edge of the main channel. From a size of 35–40 mm, an increasing number of juveniles were observed in the river channel, holding a benthic position. A diel habitat shift was also shown for individuals of all stages, between their respective daytime habitats and dead zones, where they were observed resting on the bottom in very shallow water at night. The benefits of this shift might be expressed in terms of energy conservation and (or) decreased predation risk. This study points out the importance of marginal habitats in the first weeks of grayling life as (*i*) exclusive nursery areas for larvae that use them both by day and by night and (*ii*) resting habitats for juveniles at night.

**Résumé** : Une approche basée sur des observations directes depuis la berge du cours d'eau a permis une description précise des modalités de distribution en fonction de la taille des jeunes ombres communs. Les plus petites larves (15–20 mm) sont principalement observées dans les couches supérieures de la colonne d'eau et très près de la berge. En grandissant (20–35 mm), les larves âgées et les jeunes parrs commencent à se positionner plus près du fond et de la bordure du courant. À partir d'une taille de 35–40 mm, un nombre croissant de juvéniles est observé dans le chenal de la rivière, en position benthique. Un changement d'habitat journalier a été mis en évidence pour les individus de tous stades, entre leurs habitats diurnes respectifs et les zones mortes où ils sont observés la nuit reposant sur le fond dans une très faible hauteur d'eau. Les bénéfices de ce déplacement pourraient s'exprimer en terme de conservation d'énergie et de diminution du risque de prédation. Cette étude révèle l'importance des habitats marginaux au cours des premières semaines de vie des ombres en tant que (*i*) sites nurseries exclusifs pour les larves qui les utilisent de jour comme de nuit et (*ii*) habitats de repos pour les juvéniles la nuit. [Traduit par la Rédaction]

## Introduction

As for many fish species, the life history of salmonids is characterized by a succession of habitat changes, occurring at different spatial and temporal scales, between spawning, feeding, and overwintering habitats (Nikolsky 1963; Harden Jones 1968; Northcote 1978, 1984). Beside spawning migrations, which have been intensively studied, perhaps because they are the most spectacular, seasonal changes in habitat use by stream-dwelling salmonids have also received much attention (Rimmer et al. 1983; Cunjak and Power 1986; Hillman et al. 1987; Cunjak 1988; Heggenes et al. 1991; Nickelson et al. 1992), while diel habitat shifts have been less studied (Northcote 1962; Chapman and Bjornn 1969; Harris et al. 1992). Whatever the scale considered, migrations are commonly regarded as "an adaptive phenomenon for increasing growth, survival and abundance" (Northcote 1978), perhaps the result of various trade-offs (Clark and Levy 1988). Members of the closely related Thymallinae family have received less attention, and no data exist on the diel habitat use of grayling (*Thymallus thymallus* L. 1758) early life stages. The literature is restricted to several papers describing the daytime habitat of larval and juvenile stages. After emergence from their gravel nests, larvae are found along riverbanks in shallow and slow-flowing areas, and some weeks later, juveniles are found in swifter and deeper zones in the main channel of the river (Scott 1985; Bardonnet et al. 1991). The threshold size at which the shift towards the channel occurs is not precisely known, but Scott (1985) reported that this shift coincided with the metamorphosis into the juvenile form as revealed by parr marks, which occur at a size of about 30 mm for grayling (Penaz 1975). In addition to this shift linked to ontogeny, one might expect diel changes in habitat by young grayling,

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as shown in other salmonids. Our aim in this study was thus to point out the dynamic aspect of size-related differences in day and night habitat use by young grayling in habitats for which the day use has already been described (Sempeski and Gaudin 1995*b*), but for which night use is still unknown.

## Material and methods

Field studies were conducted on the River Pollon, a small spring-fed tributary of the River Ain (France), about 80 km northeast of Lyon. This river provides optimal conditions for direct observation from the bank: the discharge is stable (mean annual discharge 1.1 $m^3 \cdot s^{-1}$, water level fluctuations ± 1 cm during the study period), the water temperature is also quite stable (from 10 to 15°C during the study period), and the water is clear throughout the year. Grayling and brown trout (*Salmo trutta*) are the two dominant fish species of the river, and some cyprinids (*Leuciscus cephalus*, *Leuciscus souffia*, and *Phoxinus phoxinus*) are also observed occasionally in some pools of the study site. We worked on a 300-m stretch, characterized by a mean width of 8.5 m (SD = 1.7, range 6.5–11.6, $n = 15$), that was used by adult grayling for spawning but was also used after emergence by larval and juvenile stages (Sempeski and Gaudin 1995*a*, 1995*b*). The two main compartments of the river successively used by larval (<30 mm) and juvenile (>30 mm) grayling are (*i*) the dead zones along the river bank, i.e., small bays created by bank curves, and (*ii*) some specific areas of the main channel. The physical characteristics of these habitats have already been described for the River Pollon (Sempeski and Gaudin 1995*b*) and also in a semi-experimental stream (Valentin et al. 1994). Dead zones were characterized by a current velocity that was usually <20 $cm \cdot s^{-1}$, a depth of <40 cm, and a substrate dominated by sand and silt. In the study site these zones were 1–2.5 m wide and 2.5–10 m long. Areas of the main channel where juveniles were observed were characterized by moderate current velocity (20–40 $cm \cdot s^{-1}$), moderate depth (40–60 cm), and a medium-sized substrate composed of gravel and pebbles. The transition zone, corresponding to the boundary between these two habitat types, constituted a third more restricted but nevertheless important compartment, clearly used by some fish.

The dynamics of day–night habitat use by young grayling were studied by assessing the fish's location in one of these physically well-defined compartments. When a fish was located in a dead zone, its position was evaluated more precisely by dividing this zone into thirds, from the bank towards the transition zone.

Water depth was measured at the fish's location, and the vertical position of the fish was assessed by visually dividing the water column into horizontal thirds (upper, middle, and lower). A fourth level identified as bottom was used to distinguish fish lying on the bottom.

Fish were located in the daytime by direct observation from the riverbank using polarizing glasses, in the early afternoon and only in sunny conditions. Each fish encountered was recorded. Daytime surface observations were more difficult for the last two surveys when juveniles were in the channel, but checks by divers immediately afterwards confirmed that the locations described from the riverbank were correct. Fish total length was visually assessed and these estimations were checked by catching some individuals with a hand net ($n = 64$). Individuals >40 mm in length were impossible to catch without electrofishing equipment and thus the estimates of their length were not checked. The mean error of the estimates ranged between 2 and 7% of fish total length, and the maximum error never exceeded 16% for any size-class. A Wilcoxon matched-pairs signed-ranks test showed no significant differences between estimated and actual sizes ($p = 0.07$). Because of the imprecision of estimates and the impossibility of checking large individuals, we classified fish between 15 and 40 mm into 5-mm classes, and fish from 40 to 59 mm into 10-mm classes, all fish ≥60 mm being pooled into a last class.

Night surveys started 30 min after the beginning of the night, i.e., after the onset of darkness. Fish were located using a hand-held flashlight, by walking slowly upstream on the riverbank or carefully in the water and randomly lighting spots along the bank and in the channel. The river channel and margins were sampled simultaneously by two observers walking in line. The light was never directed upstream and it was switched off as quickly as possible to avoid disturbing sites upstream. Thus, when fish were seen, the observers had to memorize the location and to estimate the size-class of the fish observed. Fish did not react immediately to the light, but remained motionless for a few seconds at least, and those that subsequently fled moved downstream, which reduced the risk of recording the same fish twice. Furthermore, the distance between sample sites was at least 10 m, depending on the perimeter disturbed around each site.

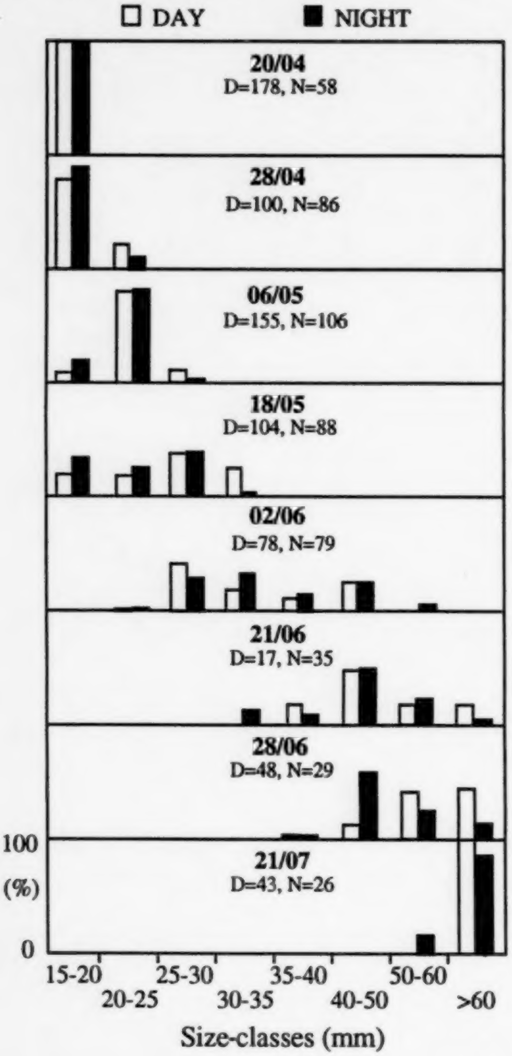
Observations began in mid-April 1993 and thereafter seven other surveys were performed, generally at 2-week intervals, until mid-July 1993 (see dates in Fig. 1).

## Results

The frequency distributions of the size-classes of fish sampled during day and night were similar (Fig. 1). On April 20, all of the fish observed belonged to the first size-class, indicating that they had all recently emerged. Thereafter, the number of size-classes rapidly increased (up to six size-classes in early June, indicating differences of 25–40 mm between the smallest and the largest individuals observed). This increasing difference in size may be explained by (*i*) a long emergence period and (*ii*) differential growth of individuals. From the end of June, the number of size-classes decreased because all of the fish >60 mm were pooled in one class. Discharge was stable during the study and therefore did not affect fish distribution.

Daytime distribution indicated a shift of grayling from the dead zone to the main channel with increasing size (Fig. 2A). The third of the dead zones nearest to the bank was only used by the smallest individuals (size-class 15–20 mm). Larger individuals were observed in dead zones, but in the third closest to the limit of the main current or on the limit itself. Above a size of 35 mm, an increasing proportion of grayling was observed in the channel, and above 60 mm most of them had left marginal

**Fig 1.** Frequency distribution of grayling size-classes observed at different dates in the River Pollon. *D* and *N* represent the number of individuals observed in daytime and nighttime, respectively.



habitats. During the night, the horizontal distribution was clearly different, individuals of all size-classes being mainly observed in dead zones (Fig. 2B). The smallest individuals (between 15 and 20 mm) were mainly found in the first third of the dead zones, close to the riverbank, while fish between 20 and 50 mm were regularly distributed from the bank to the limit of the channel. Some fish larger than 40 mm were observed in the channel at night.

The vertical distribution of grayling also differed between day and night. All fish observed in the daytime (Fig. 3) were feeding in the water column, sometimes holding a position very close to the bottom but never lying on it. Fry between 15 and 25 mm were found in the entire water column, with about half of them in the upper or the middle third. Thereafter, individuals were mainly observed in the bottom two thirds of the water column (25–35 mm) or only in the lowest third (>35 mm). During the night, all fish were resting on the bottom.

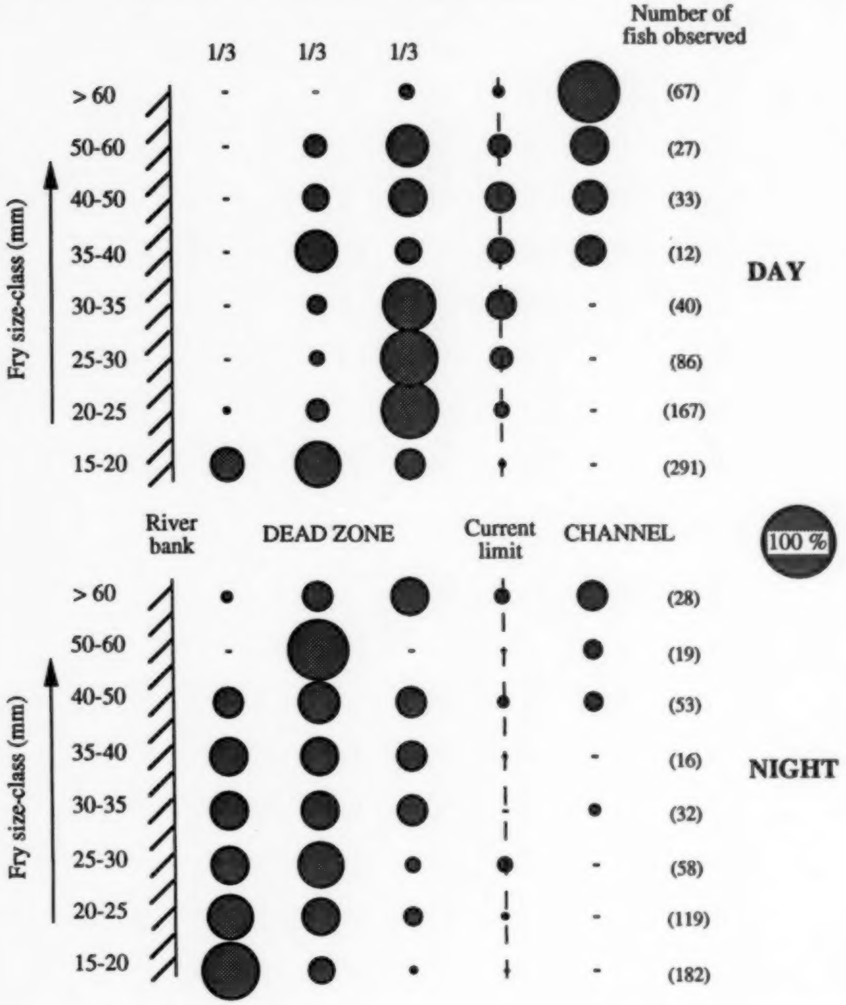
In the daytime, fish were observed in deeper water than during the night, which was related to their horizontal distribution (Fig. 4). In the daytime, fish (even those belonging to the smallest size-class) were never observed in depths <5 cm, and those larger than 20 mm were never found in less than 10 cm of water. Inversely, shallow depths were intensively used at night, as 70% of the 15–20 mm long individuals were found in depths <5 cm, and the same percentage of the 20–30 mm long fish were observed in depths <10 cm. Though the modes of preferred depths increased with fish size, the majority of the last size-class was observed in depths from 40 to 60 cm in the daytime, while still found in depths <40 cm at night (Fig. 4).

## Discussion

The general pattern of habitat use by young grayling, which is characterized by (*i*) a general tendency to shift towards the channel with increasing size and (*ii*) a diel migration between feeding and resting habitats, can be interpreted as a succession of four phases. The first phase concerns the size-class 15–20 mm, which appeared to be most linked to dead zones. Fish belonging to this group were mainly observed in the upper layers of the water column in the daytime, and at night they were found on the bottom of extremely shallow zones (often <5 cm), very close to the banks. One reason for such a marginal location is a high vulnerability to downstream displacement by flow because of the limited swimming capacity of young grayling (Scott 1985) as has been shown for other young salmonids (Heggenes and Traaen 1988). Fish from 20 to 35–40 mm represented a second homogeneous group also linked to dead zones, but to a lesser extent. The main characteristic of this group was a tendency, especially in the daytime, to adopt a position close to the bottom and to use the transition zone between dead zones and the main current of the river channel. One can hypothesize that this location enables young grayling to have a view of prey both drifting in the channel and entering the dead zone, while remaining protected from high current velocity. The third group includes individuals larger than 35–40 mm. At this length, all of the fish have reached the parr stage, as revealed by their parr marks, and are able to live in the channel. Though they still used dead zones, mainly at night, an increasing proportion of individuals was observed in the channel, and above a length of 60 mm all but a few young grayling had left dead zones in the daytime. These large individuals could form a fourth group of large parr, as their nighttime distribution also differed from that of other size-classes.

The habitat requirements of young grayling vary greatly between day and night, even though the movements involved concern small distances (a few centimetres for larvae, a few metres for juveniles). Such a shift towards marginal zones at night in streams has been reported for rainbow trout (*Oncorhynchus mykiss*) (Northcote 1962; Chapman and Bjornn 1969) and recently for brown trout

**Fig. 2.** Day and night horizontal distribution of grayling size-classes in the River Pollon.



(Harris et al. 1992) and several cyprinid species (Copp and Jurajda 1993). Diel fish migrations have also been reported in lakes either in a horizontal (Emery 1973; Keast et al. 1978) or a vertical plane (Hasler and Villemonte 1953; Gehrke 1992) or both (Gliwicz and Jachner 1992). Several hypotheses have been advanced to explain these migrations. In most cases, they have been interpreted as a trade-off between foraging behaviour and predation risk (e.g., Clark and Levy 1988). Nevertheless, in some cases, no direct evidence of the role of proximate selective forces such as prey abundance or predation risk has been found (Post and McQueen 1988). Brett (1971) proposed a bioenergetic hypothesis to explain the diel vertical migrations of juvenile sockeye salmon. He suggested that these migrations through water layers having different temperatures were a thermoregulation mechanism minimizing metabolic costs and maximizing growth. For young grayling, the major benefit of the use of dead zones may be energy saving by minimizing swimming costs in these slow-flowing areas that are well suited to the requirements of small fish such as early life stages of salmonids (Moore and Gregory 1988) and rheophilic cyprinids (Schiemer and Spindler 1989). Nevertheless, the extremely marginal location of the smallest larvae at night, in very shallow depths (<5 cm), may suggest that predation risk (especially by large brown trout, the main potential predator of young grayling in the Pollon) is also an important factor influencing the habitat selection of recently emerged individuals. Indeed, predation risk has been recognized as a main factor influencing the size-dependent spatial segregation of individuals in a fish community, with smaller individuals restricted to shallower habitats in the presence of large piscivorous fishes (Schlosser 1987; Schlosser and Angermeier 1990; Harvey 1991). Terrestrial piscivores may also influence

**Fig. 3.** Daytime vertical distribution of grayling size-classes in the River Pollon.

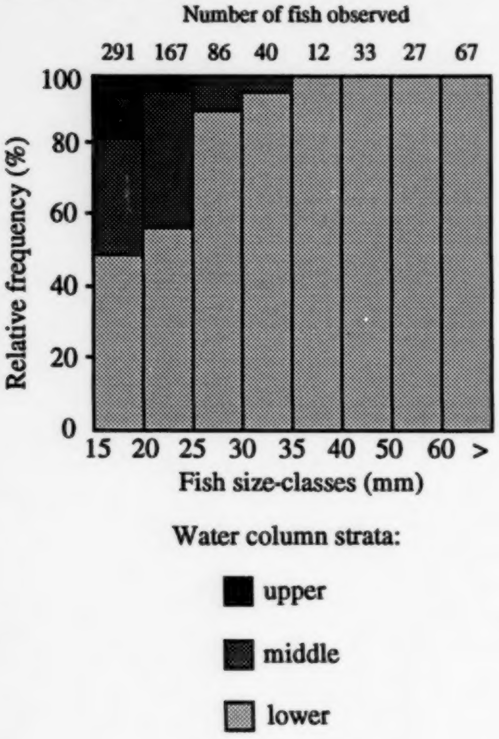
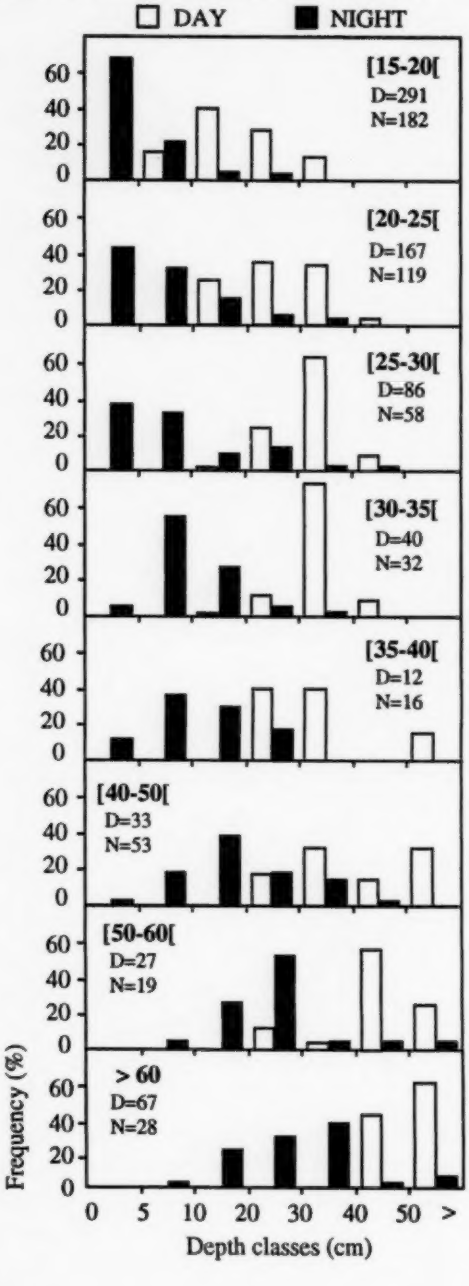


**Fig. 4.** Depth distribution of grayling size-classes in the River Pollon. *D* and *N* represent the numbers of fish observed in daytime and nighttime, respectively.



fish distribution. Power (1984) proposed that the depth distribution of loricariids, with larger individuals in deeper water, was explained by predation risk from aquatic (piscivorous fish) and also by terrestrial (bird) predators. The daytime location of larval grayling in shallow water and in the upper layers of the water column, compared with the deeper habitats selected by juveniles holding positions close to the bottom, may also reflect predator avoidance, especially since heron (*Ardea cinerea*) and kingfisher (*Alcedo atthis*), which are potential predators for young grayling, are known to occur along the River Pollon.

Though the use of lateral habitats decreases with growth, and becomes less visible as older juvenile grayling use dead zones only at night, such habitats are utilized during several months in the River Pollon, at least until the fish reach a size of about 70–80 mm (which is the size of the larger fish observed in July). The use of marginal habitats, especially at night, might be at risk as these habitats are severely affected by water level fluctuations, which can be a source of mortality or downstream displacement for small fish if these fluctuations are sudden and strong (Crisp 1991; Crisp and Hurley 1991), as may happen in regulated rivers.

The functional groups we defined do not necessarily coincide with developmental stages based on physiological and morphological cues (Penaz 1975). According to Penaz's subdivisions, our first group includes embryos (sensu Balon 1975), i.e., individuals that are still feeding on internal reserves, but also larvae, i.e., individuals feeding on external food resources, while our second group includes individuals at the transition between the larval and the juvenile stage (about 30 mm in grayling, Penaz 1975). This means that one should consider with caution the use of developmental stages (or size groups defined a priori, as often seen in the literature) as a basis for studies on fish habitat use. The notion of functional group, as defined earlier in this paper, might be a more pertinent approach and should be considered in other species. This could improve our understanding of fish–habitat relationships and provide an alternative to the use of developmental stages as the basic unit of the biological portion of present hydraulic models, especially since some groups appeared to be more sensitive than others to environmental conditions (particularly at night), because of their strict habitat requirements.

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# Spatial scales of variability in cod recruitment in the North Atlantic

**R.A. Myers, G. Mertz, and N.J. Barrowman**

**Abstract**: We reexamine the hypothesis that there are large-scale (thousands of kilometres) patterns of recruitment of Atlantic cod (*Gadus morhua*) in the North Atlantic. Previous analyses have used sequential population analysis to reconstruct population histories; however, these methods are subject to a variety of biases and rely on the accuracy of commercial catch-at-age data. Several different studies have arrived at conflicting interpretations using virtually the same data. Here we analyze alternative data from research surveys using statistical methods that explicitly account for estimation error and we also employ detrended sequential population analysis. We conclude that the spatial scale of recruitment correlations generally does not exceed 500 km.

**Résumé** : Nous réexaminons l'hypothèse selon laquelle il existerait dans l'Atlantique nord des schémas à grande échelle (milliers de kilomètres) du recrutement de la morue franche (*Gadus morhua*). Des travaux antérieurs ont eu recours à l'analyse séquentielle de la population pour reconstruire l'histoire des populations; ces méthodes sont toutefois soumises à divers biais, et sont tributaires de l'exactitude des données de la pêche commerciale sur les captures selon l'âge. Plusieurs études différentes sont parvenues à des interprétations contradictoires alors qu'elles partaient de données pratiquement similaires. Nous analysons ici d'autres données fournies par les campagnes de recherche en nous servant de méthodes statistiques qui tiennent compte explicitement de l'erreur d'estimation, et nous employons aussi une analyse séquentielle de la population épurée de la tendance temporelle. Nous concluons que l'échelle spatiale des corrélations du recrutement ne dépasse généralement pas 500 km.

[Traduit par la Rédaction]

## Introduction

A number of studies have examined large-scale patterns in recruitment in the North Atlantic (Templeman 1972; Garrod and Colebrook 1978; Koslow 1984; Koslow et al. 1987; Thompson and Page 1989; Cohen et al. 1991; Myers et al. 1995*a*). These studies are potentially important because they allow the scale of the physical and biological processes affecting recruitment patterns to be determined (Mann 1993). The degree to which recruitment is synchronous from region to region bears on the question of why different fish stocks are sometimes observed to collapse simultaneously (e.g., Koslow et al. 1987; Sinclair 1994).

These studies by and large have used sequential population analysis (SPA), which reconstructs population histories using commercial catch-at-age data. The advantage of SPA data is that it is available for the majority of important commercial fish stocks in the North Atlantic, making it particularly suitable for stock-to-stock synchrony studies. One purpose of this paper is to examine the utility and tenability of the use of SPA data in studies of this type. Drawbacks to the use of SPA data lie in the fact that spurious trends may arise for a number of reasons, e.g., incorrect estimates of natural mortality, changes in catchability, trends in misreporting, and incorrect assumptions about the probability distribution of the estimation errors (Lapointe et al. 1989; Myers and Cadigan 1995). To investigate the significance of these extraneous trends we will consider whether or not the SPA stock-pair correlations have a well-behaved dependence on stock separation. We will compare the SPA-based recruitment data with survey-based recruitment data to identify artifactual trends. Also, we will attempt to show that a very simple fit to the spatial correlation field can extract almost all utilizable information; that is, the residuals from the fit represent sampling and stock specific variability alone. We will limit our study to Atlantic cod (*Gadus morhua*) recruitment because the most reliable time series are for that species.

## Methods and data

### Sequential population analysis

We assembled all time series of spawner abundance and recruitment for cod populations in the North Atlantic (Myers et al. 1995*b*). Spawning stock biomass (SSB) and recruitment were estimated by SPA of commercial catch-at-age data. SPA techniques include virtual population analysis, cohort analysis, and related methods that reconstruct population size from catch-at-age data (Hilborn and Walters 1992). The stock boundaries in the North Atlantic generally adhere to those of the Northwest Atlantic Fisheries Organization (NAFO) and International Council for the

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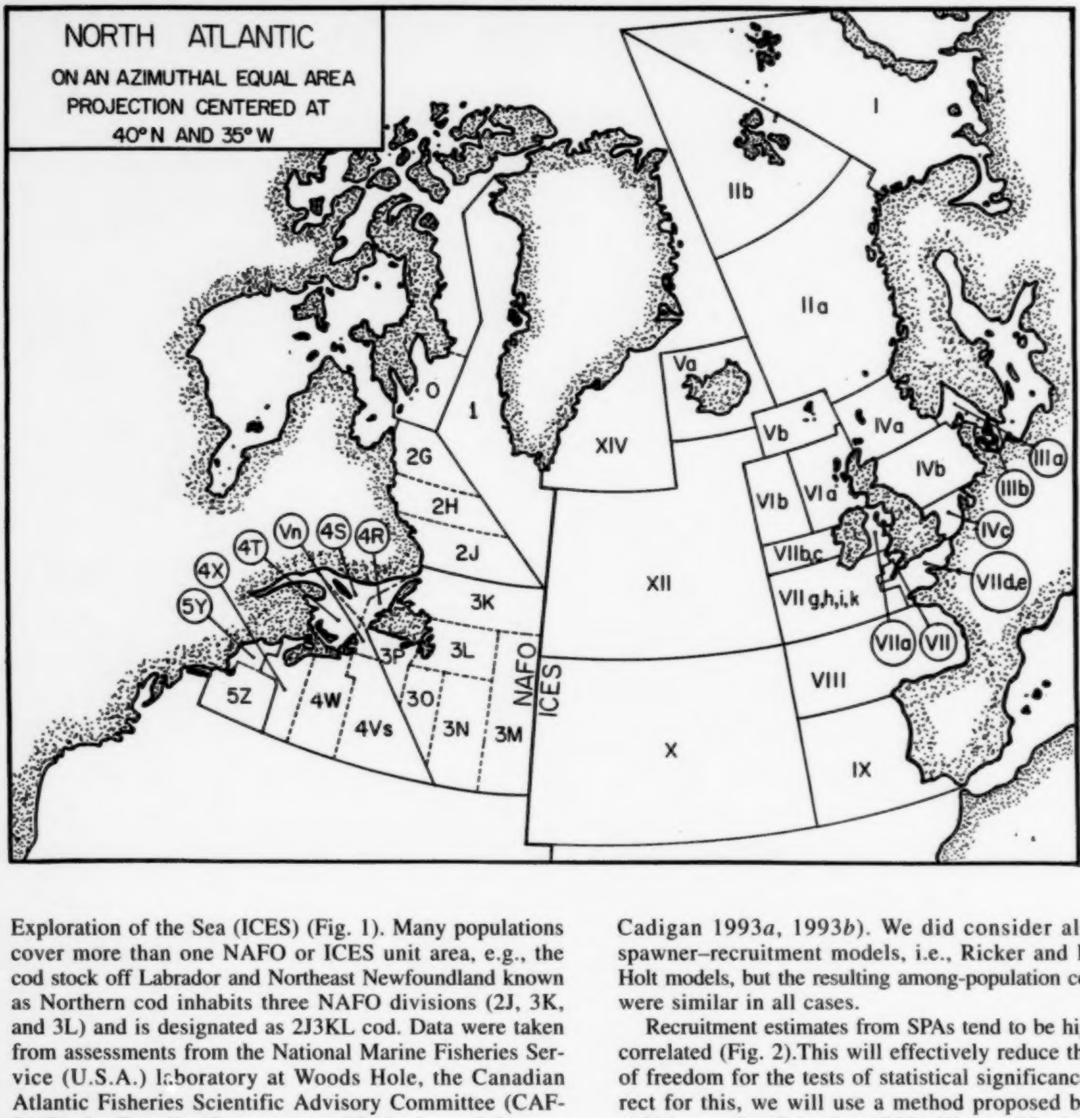
**Fig. 1.** Map of the North Atlantic showing the regions used to define populations for management.



Exploration of the Sea (ICES) (Fig. 1). Many populations cover more than one NAFO or ICES unit area, e.g., the cod stock off Labrador and Northeast Newfoundland known as Northern cod inhabits three NAFO divisions (2J, 3K, and 3L) and is designated as 2J3KL cod. Data were taken from assessments from the National Marine Fisheries Service (U.S.A.) laboratory at Woods Hole, the Canadian Atlantic Fisheries Scientific Advisory Committee (CAFSAC), the Canadian Department of Fisheries and Oceans, the NAFO, and ICES. The SSB and recruitment data are compiled in Myers et al. (1990, 1995*b*).The SPA for the Gulf of Maine was too short (9 years) to be used in the correlation analysis so we will only present the results for the research vessel series for this population.

We log transformed the recruitment and SSB estimates to stabilize variance in recruitment and to linearize the spawner–recruitment relationship. If the regression of log recruitment on log spawners was positive and $p \le 0.10$, we used the residuals from the regression. The maximum value of the slope was constrained to be 1. Such a regression implicitly assumes that density-dependent mortality is proportional to log density. This assumption appears to be reasonable for most groundfish stocks (Myers and Cadigan 1993*a*, 1993*b*). We did consider alternative spawner–recruitment models, i.e., Ricker and Beverton-Holt models, but the resulting among-population correlations were similar in all cases.

Recruitment estimates from SPAs tend to be highly autocorrelated (Fig. 2).This will effectively reduce the degrees of freedom for the tests of statistical significance. To correct for this, we will use a method proposed by Bartlett (1946; see also Priestley 1981). We modify the standard test for the correlation coefficient being different from zero by using the corrected degrees of freedom defined by

$$N' = N\frac{(1 - a_1 a_2)}{(1 + a_1 a_2)}$$

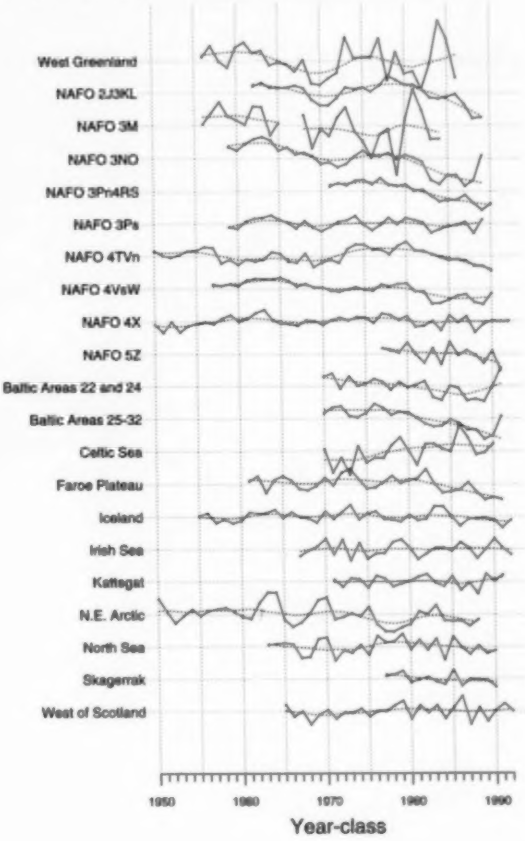
where $N$ is the number of pairs of observations in the two series, $N'$ is the adjusted degrees of freedom assuming an AR(1) process, and $a_1$ and $a_2$ are the lag one autocorrelations. Note that the above equation can give estimates of the effective number of degrees of freedom greater than the number of data points. Even though this is statistically valid if there are negative autocorrelations (Priestley 1981), we will constrain our estimates such that $N' \le N$, to be conservative and because no estimates of negative autocorrelations were statistically significant. This method will not account for low frequency variability in the time series that are not well described by a first-order autoregressive process. There is a fundamental difficulty with estimating the correct degrees of freedom in this case because it is not possible to estimate the statistical properties of low frequency variability from short time series. Thus, we will overestimate $N'$ if there is low frequency variability in the time series.

### Research survey estimates

In our analysis of the SPA estimates of recruitment we use the standard product moment estimates of correlation. However, there are large estimation errors in the research survey estimates of recruitment that would make the estimated correlation biased. That is, the correlations could not be easily interpreted. We develop statistical models to estimate the correlations in recruitment between populations by using research surveys that explicitly model estimation errors. The model estimates the correlation in recruitment between two populations, the underlying variance in recruitment, and the estimation error variance of each survey by fitting the observed variances of research surveys and covariances between surveys carried out on two populations using maximum likelihood. These methods are based upon confirmatory factor analysis models, which are structural equation models (Bollen 1989). This approach is similar to that of Myers and Cadigan (1993*a*, 1993*b*); the methods are developed in the Appendix.

We attempted to compile and analyze all data sets in the Northwest Atlantic for which there were reliable research survey estimates of juvenile fish abundance. We used data from surveys based upon a stratified random design (Doubleday and Rivard 1981). For three surveys, there were zero catches in three cases: age 1 fall surveys in the Gulf of Maine, age 2 Canadian surveys on St. Pierre Bank, and age 0 fall surveys of Georges Bank. We replaced zeros with the minimum estimated for that age in these time series; a total of 6 zero estimates were modified by this procedure. Details of the surveys are given in Table 1. For three populations there were no reliable time series of research surveys that could be used. For West Greenland and Flemish Cap the research vessel series were less than 10 years, while for the Northern Gulf of St. Lawrence the time series for the winter surveys had estimation error that was too large to produce reliable estimates, i.e., the maximum likelihood estimates failed to converge in the feasible part of parameter space in most cases. For West Greenland and the Northern Gulf of St. Lawrence we used the SPA estimates of recruitment, instead of the research surveys, in the analysis described in the appendix. To test the robustness of our conclusions, we attempted to use more than one survey whenever possible. In one case, the 3L spring survey, the survey covered only part of the range of the population.

**Fig. 2.** Recruitment time series from sequential population analysis (SPA) corrected for spawner biomass for populations analyzed (solid lines). Estimates of numbers-at-age in a year-class are $\log_{10}$ transformed with the mean subtracted. The mean of each series is separated by 1 unit, i.e., a factor of 10, from the one below. The smooth was obtained by using the Lowess filter with a 10-year window and is given by the broken line.



We display recruitment estimated from the research surveys listed in Table 1 under the assumption that the error variances are constant for all surveys (Fig. 3). This is equivalent to using the multiplicative model of Shepherd and Nicholson (1991), which assumes that research survey estimates of numbers-at-age are a multiplicative function of a cohort effect and a catchability-at-age effect. This method, which was also used by Myers et al. (1993), is described as follows. Let the number of fish of age $a$ in year $y$ be $N_{y,a}$ and consider only ages at which commercial fishing mortality is low. Let $P_a$, the survival to age $a$, be constant. Let the number of recruits in year $y$ be $N_{y,0}$. The numbers-at-age will then be

$$N_{y,a} = P_a N_{y-a,0}$$

Let the number of fish of age $a$ in year $y$ estimated from survey $i$ be $R_{y,a,i}$. Assume that the estimate of abundance is proportional to the true abundance, i.e., the catchability of survey $i$ at age $a$, is $Q_{a,i}$. The error, $\delta_{y,a,i}$, appears in the model multiplicatively. Thus,

$$\begin{aligned} R_{y,a,i} &= Q_{a,i} N_{y,a} e^{\delta_{y,a,i}} \\ &= Q_{a,i} P_a N_{y-a,0} e^{\delta_{y,a,i}} \end{aligned}$$

**Table 1.** Research surveys from the Northwest Atlantic.

| Population | NAFO Div. | Country | Season | Ages | Years | Missing years |
|---|---|---|---|---|---|---|
| Labrador – Northern Grand Banks | 2J3KL | Canada | Fall | 3–5 | 1978–1993 | — |
|  | 3L | Canada | Spring | 3–5 | 1977–1993 | 1983–1984 |
| Southern Grand Banks | 3NO | Canada | Spring | 3–5 | 1971–1993 | 1983 |
|  | 3NO | Russia | Spring | 3–5 | 1977–1993 | 1992 |
| St. Pierre Bank | 3Ps | Canada | Spring | 3–5 | 1972–1994 | — |
|  | 3Ps | France | Spring | 3–5 | 1978–1991 | — |
| Northern Gulf of St. Lawrence | 3Pn4RS | Canada | Winter | 3–5 | 1978–1994 | 1982 |
| Southern Gulf of St. Lawrence | 4TVn | Canada | Summer | 3–5 | 1971–1993 | — |
| Eastern Scotian Shelf | 4VsW | Canada | Spring | 2–4 | 1979–1994 | 1985 |
|  | 4VsW | Canada | Summer | 2–4 | 1970–1993 | — |
| Browns Bank | 4X | Canada | Summer | 3–5 | 1970–1993 | 1988 |
| Gulf of Maine | 5Y | U.S.A. | Spring | 1–3 | 1968–1992 | — |
|  | 5Y | U.S.A. | Fall | 1–3 | 1963–1991 | — |
| Georges Bank | 5Z | U.S.A. | Spring | 1–3 | 1968–1994 | — |
|  | 5Z | U.S.A. | Fall | 0–2 | 1963–1993 | — |

**Note**: Ages and years refer to minimum–maximum ages and first–last years used in the analyses.

where $\delta_{y,a,i}$ is assumed to be normal with constant variance. Using a log transformation, and letting lowercase letters represent the log transform of parameters and variables, the last equation becomes

$$[1]\quad r_{y,a,i} = q_{a,i} + p_a + n_{y-a,0} + \delta_{y,a,i}$$

Note that $q_{a,i}$ and $p_a$ are confounded in the above equation, and therefore we only attempt to estimate the sum of these two parameters. The estimates of recruitment we display in Fig. 3 are the $n_{y-a,0}$ in the above equation.

Population to population correlations can incorporate, in a systematic manner, multiple research surveys of a population (Table 1) by using methods described in the Appendix. Note that in the estimation of the underlying correlation we do not assume that surveys at different ages have the same estimation error variance as we did when we plotted the recruitment estimated from the surveys above. The correlations we estimate from the research surveys are unlike the standard product moment Pearson correlations in several ways. Firstly, they do not necessarily lie between −1 and 1, because they are derived parameters. Secondly, the standard correlations calculated from data with measurement errors will be biased towards zero, i.e., a small correlation may be estimated simply because both time series are measured with errors. This is particularly a problem with the research surveys that usually have large estimation errors, but the procedure described in the appendix largely eliminates this problem. Cadigan (1993) showed, using simulations, that this procedure is almost unbiased as long as the data series cover more than 10 years and the estimation error variance is less than the variance of the underlying random variable. However, the estimated correlation will have a high variance because several parameters are estimated to estimate the correlation.

### Estimating spatial scale

The spatial scale is estimated as the distance over which the correlation in recruitment is reduced by a factor $e^{-1}$, i.e., the exponential decay rate or the *e*-folding scale. That is, we fit the model:

$$[2]\quad \rho(d) = \rho_0 e^{-\nu d}$$

where $\rho_0$ is the correlation at a distance of zero, $\nu$ is the *e*-folding scale, and $d$ is the great circle distance between spawning locations of each population in kilometres. This equation was fitted using nonlinear least squares weighted by the number of common years used to estimate the correlation. We did not obtain a significantly better fit when we estimated $\rho_0$, and therefore we will report only the fits with $\rho_0$ constrained to be one. The above model is a simple AR(1) process. We also fit models with an AR(2) process with distance, but none was significant.

The above model assumes isotropy and homogeneity. While the assumptions are not true, they are probably a reasonable approximation for much of the data because most of the stocks are on relatively straight continental shelves. We will not deviate from our simple model in the text.

An alternative approach to ours is to use pricipal component or canonical correlation analysis. These methods may be more powerful in detecting a signal (Thompson and Page 1989), but do not lead to a simple *e*-folding scale that is easy to interpret.

### Short-term versus long-term variability

We separated the variability in each recruitment series from SPA into two components: low frequency (periods greater than 10 years) and higher frequency variation. We estimated the low frequency variation using a smoother known as Lowess (described by Cleveland 1979). Lowess uses robust locally linear fits within a time window, 10 years in our case, around each recruitment year. Recruitment estimates that are inside the window are weighted so that nearby points get the most weight. The higher frequency variation was defined as the residuals from the smoothing (Fig. 4). As will be shown, similar results were

**Fig. 3.** Recruitment time series estimated from research surveys for the Northwest Atlantic using a multiplicative model on the ages shown on the right. Estimates of numbers-at-age in a year-class are $\log_{10}$ transformed with the mean subtracted. The mean of each series is separated by 2 units, i.e., a factor of 100, from the one below. The first and last year-classes are estimated using only one survey each; the corresponding estimates are indicated by broken lines and open circles.

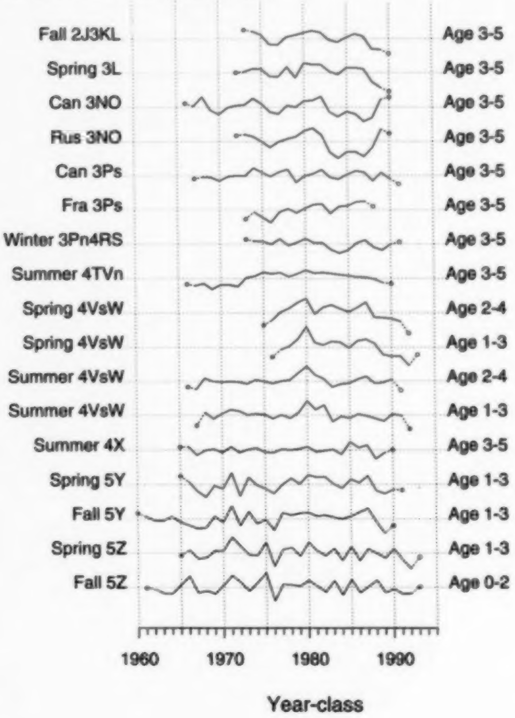
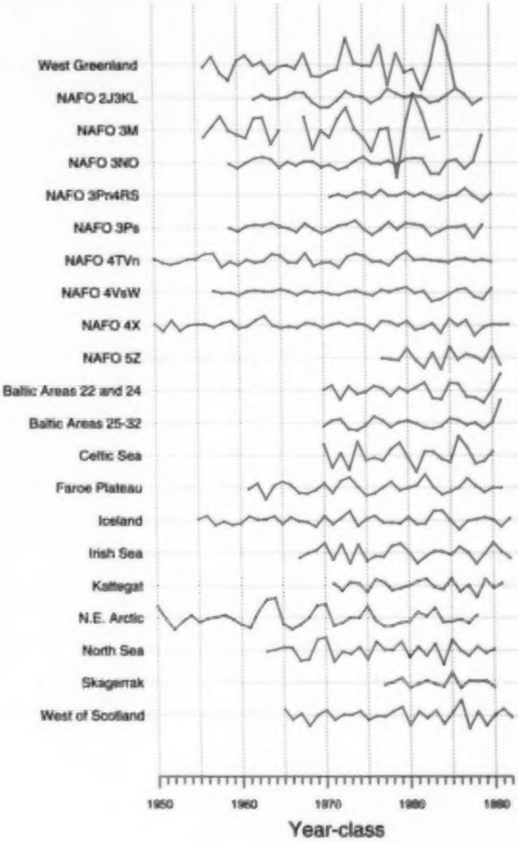


**Fig. 4.** Residuals of the recruitment from SPA corrected for spawner biomass, and with the long-term trend shown in Fig. 2 removed. Residuals are $\log_{10}$ transformed with the mean subtracted. The mean of each series is separated by 1 unit, i.e., a factor of 10, from the one below.



obtained when a smaller (5 years) or larger (15 years) window was used.

Some previous studies have used first differencing to remove the long-term variability from the time series (Thompson and Page 1989; Cohen et al. 1991). While this is an appropriate method for many recruitment time series because they approximate simple random walks, it is not appropriate for many others. For example, recruitment in the North Sea has a negative first-order autocorrelation. We therefore use a more flexible approach.

## Results and discussion

Before presenting our correlation analysis for the North Atlantic in toto, the Northwest Atlantic, and the Northeast Atlantic, we will discuss our rationale for removing the low frequency component of the SPA data.

### Short-term versus long-term variability

A crucial question is how much of the long-term variability in the SPA results is real and how much is an artifact of assumptions, trends in misreporting, and statistical bias. Many of the trends seen in the SPA are not seen in the research survey estimates (Fig. 3). This is particularly evident in the Northern Gulf of St. Lawrence (NAFO Div. 3Pn4RS) and the eastern Scotian Shelf (4VsW) where the large declines in the 1980s in the SPA are not apparent in the research surveys.

We will demonstrate the importance of separating the SPA estimates of recruitment into long-term trends and shorter-period residuals by comparing estimated recruitment at age 3 from the research surveys and SPA in NAFO Div. 3Pn4RS (Northern Gulf of St. Lawrence). The Northern Gulf of St. Lawrence population shows apparent high correlations with several European populations if the long-term trends are not removed (Table 2). We apply the same Lowess smoother to both time series and compare the long- and short-term variability (Fig. 5). The short-term variability is simply the residuals from the long-term Lowess trend. The match between the short-term trends is excellent, and relative year-class strength is clearly seen in both time

**Table 2.** Correlations (×100) for SPA of (*i*) log(recruitment) corrected for log(spawners) (above

| | West Greenland | 2J3KL | 3M | 3NO | 3Pn4RS | 3Ps | 4TVn | 4VsW | 4X | 5Z |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — | 13 | −5 | −7 | −15 | −2 | 15 | −8 | 10 | 2 |
| 2J3KL | 1 | — | 16 | 57 | 71 | 51* | 62 | 69 | 46* | 34 |
| 3MVPA | −7 | 28 | — | 25 | −8 | 43 | −18 | 20 | 9 | −11 |
| 3NO | −41* | 16 | 24 | — | 61 | 54* | 13 | 82* | 42* | 7 |
| 3Pn4RS | −36 | 22 | 13 | −24 | — | 34 | 76 | 84 | 56* | 41 |
| 3Ps | −11 | 56† | 48* | 39* | 21 | — | 36 | 52* | 48 | 8 |
| 4TVn | 16 | 26 | −6 | 19 | 25 | 40* | — | 25 | 5 | 27 |
| 4VsW | −24 | 41* | 9 | 2 | 71† | 54† | 38* | — | 45* | 39 |
| 4X | −10 | 33 | 13 | 8 | 52* | 51† | 2 | 39* | — | 61* |
| 5Z | 0 | 0 | −8 | −9 | 32 | −1 | 13 | 26 | 57* | — |
| BA2224 | −17 | 20 | 6 | −6 | 17 | 1 | 15 | 47* | 11 | −6 |
| BA2532 | −2 | −14 | −26 | −13 | 14 | −27 | −28 | 18 | 10 | 0 |
| CS | −42 | 27 | −50 | −15 | 33 | 10 | 8 | 38 | 23 | −5 |
| FAPL | −31 | 11 | 39 | −21 | 41 | 25 | −3 | 32 | −8 | −11 |
| ICE | 45* | −32 | −2 | −19 | −38 | −23 | 5 | −43* | −14 | 14 |
| IS | −23 | 7 | −44 | −16 | 9 | 20 | −14 | 28 | 31 | 13 |
| KAT | 13 | −1 | 31 | 28 | −10 | 30 | 12 | 3 | 23 | −2 |
| NEAR | −20 | −25 | 9 | 31 | −8 | 1 | 17 | −10 | 9 | 2 |
| NS | −17 | −21 | −32 | −3 | −5 | −23 | −24 | 5 | 1 | 49 |
| SKAG | 17 | 6 | −43 | 13 | −11 | −3 | −21 | −15 | 17 | 11 |
| VIa | −32 | 14 | −22 | −21 | −10 | −20 | −20 | 8 | −13 | 0 |

**Note**: Coded names for the populations are given in the first column. The horizontal and vertical gaps through the

*$p < 0.05$, corrected for autocorrelation.

†$p < 0.01$, corrected for autocorrelation.

series; however, the long-term trends are clearly different. The decline seen in the SPA is not seen in the research surveys. We believe that the decline in recruitment seen in the SPA does not represent the true variability of age 3 fish but is caused by trends in misreporting (see conclusions). Similar differences in trends are seen in other Canadian time series and appear to be caused by discarding and nonreporting of young fish in recent years (Myers et al. 1995*c*).

We will concentrate on the short-term variability of recruitment because this is the only portion that is reliable and can be interpreted statistically. We will return to the long-term variability in the conclusions.

### North Atlantic SPA results

All possible correlation coefficients for pairwise comparisons of populations were calculated for the North Atlantic cod stocks (Table 2). If these coefficients are to be accepted at face value they must show a plausible dependence on separation between stocks, presumably a decay with distance. We plotted these coefficients versus distance; the behavior is anomalous in that some of the strongest correlations are found for the largest separations (Fig. 6*a*). This finding is reinforced by inspection of the residuals from an exponential fit to correlation versus distance (Fig. 6*b*). The pattern of residuals is certainly unacceptable.

The failure of the correlation coefficients to decay with distance stems from long-term trends (indicated by the broken lines) in a number of series (Fig. 2). This is especially clear for the case of the two Baltic stocks, which, owing to trends, are strongly correlated with stocks such as 3Pn4RS, 4VsW, and 3NO, which exhibit similar trends. Cohen et al. (1991) also emphasized the importance of trends in producing (perhaps) spurious correlations between recruitment series (cod and haddock stocks of the Northwest Atlantic in their study). Moreover, Myers et al. (1995*a*) found no evidence of long-distance correlations in their study of recruitment series of the North Atlantic; they partially removed trends by dividing the time series into two portions, a prediction segment (1950–1971) and a verification segment (1972–1993). The unacceptable pattern of correlations corroborates our conclusion above that the trends must be removed (Fig. 6*b*).

The correlation analysis was repeated, using the residual series (Fig. 7*a*). It is apparent that the cluster of unacceptably strong correlations at large separations has disappeared. The solid line represents an exponential fit to the decay of the correlation with distance; the *e*-folding scale is about 400 km. The residuals from the exponential fit are also displayed (Fig. 7*b*). There is a group of negative correlations at spatial scales around 2500 km. These negative correlations are associated with West

**diagonal), and (*ii*) residuals from smooth of log(recruitment) corrected for log(spawners) (below diagonal).**

| Baltic | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Areas 22 and 24 | Areas 25–32 | Celtic Sea | Faroe Plateau | Iceland | Irish Sea | Kattegat | Northeast Arctic | North Sea | Skagerrak | West of Scotland |
| −27 | −9 | −21 | −30 | 34 | −24 | 5 | −13 | −8 | 13 | −11 |
| 44 | 55 | 4 | 42 | 0 | 2 | 12 | −10 | 20 | 33 | 28 |
| 3 | −26 | −41 | 40 | −6 | −38 | 30 | 29 | −31 | −41 | −18 |
| 56 | 51 | −35 | 16 | −5 | −9 | 38 | 32 | 12 | 33 | −7 |
| 65* | 87 | −36 | 68 | 9 | −12 | 7 | −11 | 15 | 43 | 1 |
| 7 | 5 | −3 | 34 | −4 | 12 | 30 | 9 | −10 | 18 | −13 |
| 37 | 56 | −15 | 41 | 15 | −23 | 18 | −8 | 14 | 34 | 10 |
| 71* | 71 | −14 | 45 | −8 | 10 | 13 | 19 | 16 | 31 | 2 |
| 22 | 41 | 2 | 14 | −4 | 26 | 14 | 1 | 14 | 40 | −7 |
| −15 | 28 | −5 | 32 | 22 | 8 | −18 | −24 | 56* | 30 | 2 |
| | | | | | | | | | | |
| — | 65* | −41 | 20 | −22 | −8 | 44* | 16 | 21 | 35 | 28 |
| 62† | — | −40 | 45 | 9 | −6 | 6 | −14 | 26 | 50 | 13 |
| −5 | −12 | — | −20 | −42 | 68† | −28 | −21 | 25 | −19 | 38 |
| 3 | −22 | 17 | — | 31 | −3 | 2 | 0 | 19 | 35 | 6 |
| −49* | −36 | −33 | −2 | — | −18 | −6 | 25 | −9 | −16 | −33 |
| −15 | 1 | 83† | 7 | −15 | — | −18 | −4 | 27 | −13 | 23 |
| 40 | 7 | −29 | 7 | −4 | −20 | — | 2 | 15 | 28 | −18 |
| −4 | −43 | 1 | −15 | 14 | −4 | −16 | — | 9 | −38 | −5 |
| 21 | 16 | 27 | 14 | −10 | 32 | 15 | 26 | — | 66† | 62† |
| 17 | 5 | −13 | 5 | −11 | −19 | 37 | −6 | 54* | — | 15 |
| 42* | 23 | 39 | 14 | −38* | 27 | −24 | 17 | 59† | 6 | — |

middle of the table indicate the partitioning of the stocks into the Northwest Atlantic and Northeast Atlantic groups used in the analysis.

Greenland and Iceland (Fig. 7*b*). These two populations are not independent from each other because there is an important migration between them (Jakobsson 1992). Furthermore, recruitment between the two populations is significantly positively correlated (Table 2). There are four nominally significant negative correlations between these populations and other populations on both sides of the Atlantic in the detrended data series (Table 2). These are the only such negative correlations found. It is difficult to assess the statistical significance of these correlations because the populations on either side of the Atlantic are not independent, but we shall return to the issue in the conclusion.

The spatial correlation resulting from application of the 5- and 15-year cutoff-period Lowess filters is not influenced by the cutoff period (Figs. 7*c*, 7*d*). Since the 10-year filter best seemed to capture our conception of the trend, we have opted to emphasize the residuals from this smoothing.

We applied the $z$-transform

$$z(r) = \frac{1}{2} \ln \frac{1+r}{1-r}$$

to the correlation coefficients (Fig. 7*b*) and computed their cumulative distribution (Fig. 8). The solid line shows the theoretical distribution of $z$ assuming that the true correlation between any two time series is zero, in which case $z \sim \mathcal{N}(0,(N'-3)^{-1})$, where $N' = 21$ is the number of degrees of freedom of the analyzed series (we have used an $N'$ equal to the median of the $N'$s for the stock-pairs shown in Fig. 7*b*) (Sokal and Rohlf 1981). The theoretical distribution fits the data well (Fig. 8), suggesting that the exponential fit (Fig. 7*a*) has captured most of the true correlations in the data, i.e., those not arising from finite sample size.

## Northwest Atlantic

The exponential fit to the decay of correlation with distance for the Northwest Atlantic cod stocks alone shows that the $e$-folding scale is about 460 km (Fig. 9). This scale may be compared with that of plausible physical forcing agents.

The correlations estimated from the research surveys (Table 3, Fig. 10) generally exhibit the same pattern as those from the SPA. This is an important result, because it shows that the results are not an artifact of the SPA. The correlation scale is about 750 km for this set of entries, somewhat, but not greatly, different than that based on the SPA series. It must also be noted that there are unremoved trends in the survey data that may inflate the correlation scale.

It may be of consequence that recruitment in 3Ps is correlated with that in 2J3KL, 4VsW, and 4X (we assume that a correlation greater than about 0.5 merits attention). This suggests some covariation of stocks on the Newfoundland Shelf with those on the Scotian Shelf, although one might expect that these two regions are distinct environments. In fact, recent analyses indicate that there is appreciable covariation of some Newfoundland and Nova Scotia shelf water characteristics. Thompson et al. (1988) found that the first principal component of sea surface temperature (SST),

**Fig. 5.** The long- and short-term recruitment variability for Northern Gulf of St. Lawrence (NAFO Div. 3Pn4RS) cod for the SPA (broken line) and research survey at age 3 (solid line). The long-term variability is estimated using the Lowess smoother with a 10-year window and the short term is the residuals from the observed and the smooth. Estimates of numbers-at-age in a year-class are $\log_{10}$ transformed with the mean removed. The mean of each series is separated by 1 unit, i.e., a factor of 10, from the one below.

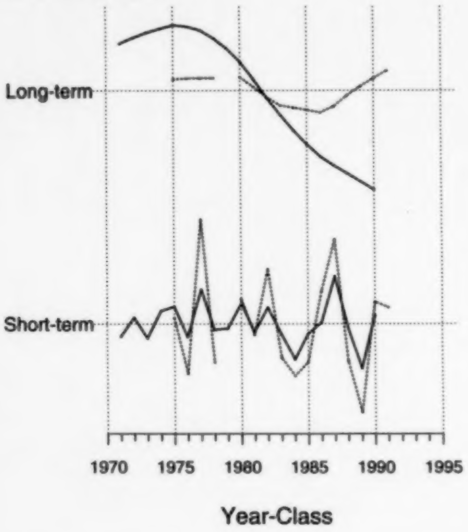
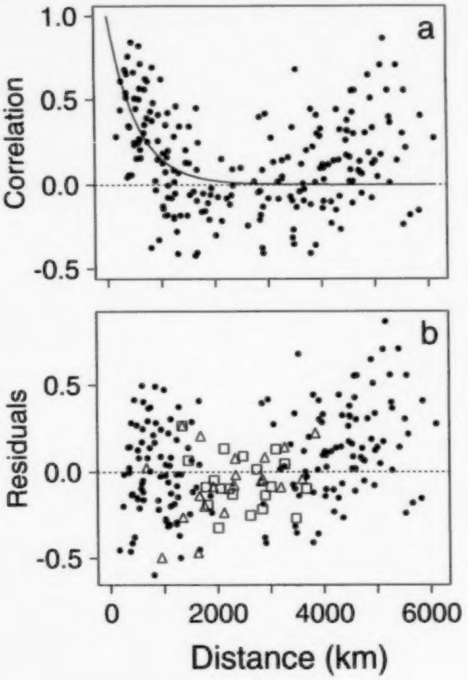


**Fig. 6.** (*a*) Correlation of recruitment, corrected for spawner stock biomass (SSB) from SPA, versus distance for cod stocks in the North Atlantic. The estimated decay parameter is $\hat{\theta} = 0.0019$ km$^{-1}$ (SE = 0.00018), i.e., an *e*-folding distance of 526 km. (*b*) Residuals from *a*. Correlations with Iceland are identified by triangles and correlations with Greenland by squares.



which is the dominant component at periods of 1–10 years, captures much of the variability on both the Grand Banks and the Scotian Shelf. The SST variability corresponds to the wind field (Thompson et al. 1988) and the first principal component of atmospheric pressure has a similar pattern to that of the SST (Koslow et al. 1987). From the site to site correlation of annual air temperature anomalies versus separation distance between maritime weather stations for the Northwest Atlantic (Fig. 11) it is evident that the decorrelation scale is on the order of 1500 km, a result consistent with Thompson et al. (1988).

There are environmental variables with spatial correlation scales equal to or greater than the correlation scale for recruitment of Northwest Atlantic cod. Since recruitment is determined, presumably, by a number of processes acting on different prerecruit stages, the scale for recruitment will probably be less than that for the largest scale environmental variables, since some of the influences on recruitment are likely to be typified by smaller spatial scales.

In summary, among the Northwest Atlantic cod stocks there are few pairs of stocks (11) that are significantly positively correlated, after the recruitment data are detrended. The unexpected correlation between West Greenland (NAFO Div. 1) and Labrador – Northern Grand Banks (NAFO Div. 2J3KL), reported by Koslow (1984), is not apparent in the detrended SPA data or the survey data examined here; evidently the result reported by Koslow was caused by data trends. Our results for the Northwest Atlantic are consistent with those of Thompson and Page (1989) and Cohen et al. (1991). The detrended SPA analysis identifies the stocks 2J3KL, 3Ps (St. Pierre Bank), and 4VsW (Eastern Scotian Shelf) as a shelf complex, showing appreciable similarity in recruitment histories.

### Northeast Atlantic

The Northeast Atlantic subset of correlations (Fig. 12) again provides a plausible fit to the exponential decay model of correlation with distance. The *e*-folding scale is about 340 km, somewhat less than that calculated for the SPA-based correlations for the Northwest Atlantic, although the two are not significantly different. As was the case for the Northwest Atlantic, the interannual anomalies in the windfield tend to have very large spatial scales (see, for example, Thompson and Hazen 1983), providing an agent capable, in principle, of imparting broad-scale coherence to the recruitment data. Although, again, we cannot identify the specific forcing responsible for establishing covariation of recruitment between stocks, we can be certain that there are environmental variables having sufficiently large scale to impose this coherence.

The correlation of recruitment between the Celtic Sea and the Irish Sea is the strongest correlation found among the detrended series. Detrending actually increases the

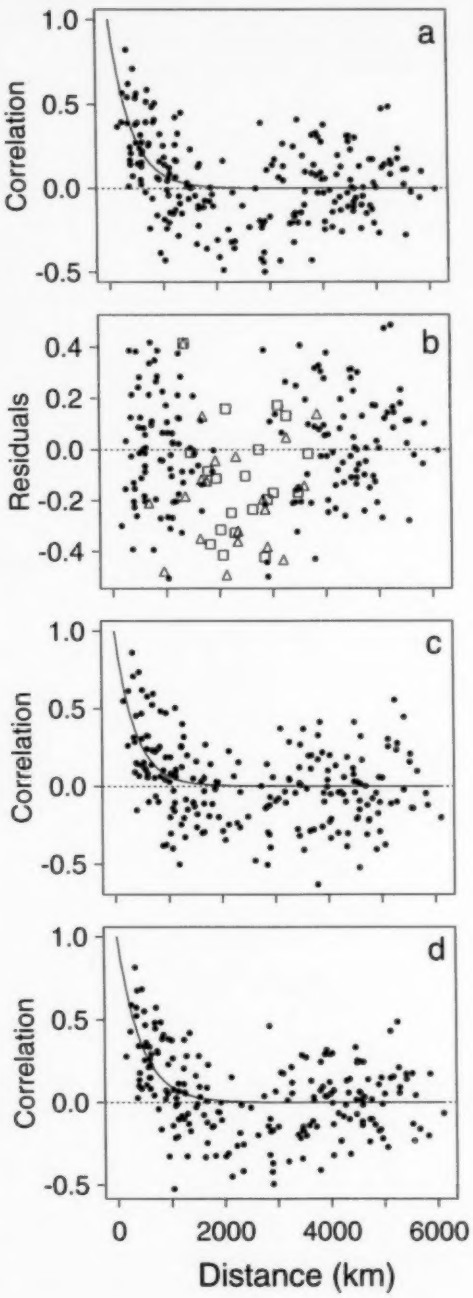
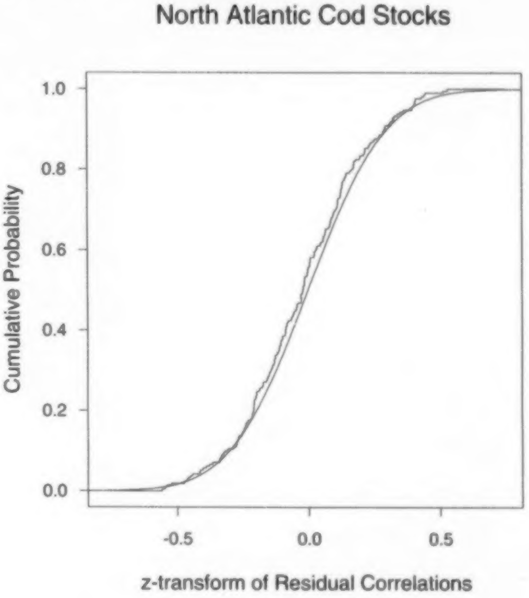


**Fig. 7.** (*a*) Correlation of residuals from the smooth of recruitment with a 10-year window, corrected for spawner biomass, from SPA versus distance for cod stocks in the North Atlantic. The estimated decay parameter is $\hat{\nu} = 0.00247\ \mathrm{km}^{-1}$ (SE = 0.00022), i.e., an *e*-folding distance of 405 km. (*b*) Residuals from *a*. Correlations with Iceland are identified by triangles and correlations with West Greenland by squares. (*c*) Correlation of residuals from the smooth of recruitment with a 5-year window, corrected for spawner biomass. (*d*) Correlation of residuals from the smooth of recruitment with a 15-year window, corrected for spawner biomass.

**Fig. 8.** The theoretical (smooth curve) and observed (jagged line) cumulative probability distribution for the *z*-transform of the residuals from Fig. 6*b*.



correlation between these two series from 0.68 (prior to detrending) to 0.83. The reason for this is evident from a comparison of Figs. 2 and 4. The cod recruitment series for the Celtic Sea possesses a pronounced low frequency trend not shared with the series for the Irish Sea (Fig. 2). The two detrended series show exceptional visual coherence, matching one another peak by peak (Fig. 13). This case suggests that there are two different processes at work, one driving recruitment variations, common to both series, with a period of about 5 years, and a long-term influence that is manifested only in the Celtic Sea.

## Conclusions

The importance of careful removal of trends in recruitment data is evident from our study. The untreated SPA data produce unacceptable spatial patterns of stock-pair correlations (Figs. 6*a*, 6*b*). It is apparent, through comparisons of survey-based and SPA-based series, that many of the trends

**Fig. 9.** Correlation of residuals from the smooth of recruitment, corrected for spawner biomass, from SPA versus distance for cod stocks in the Northwest Atlantic. The estimated decay parameter is $\hat{\theta} = 0.00217\ \mathrm{km}^{-1}$ (SE = 0.00029), i.e., an *e*-folding distance of 461 km.

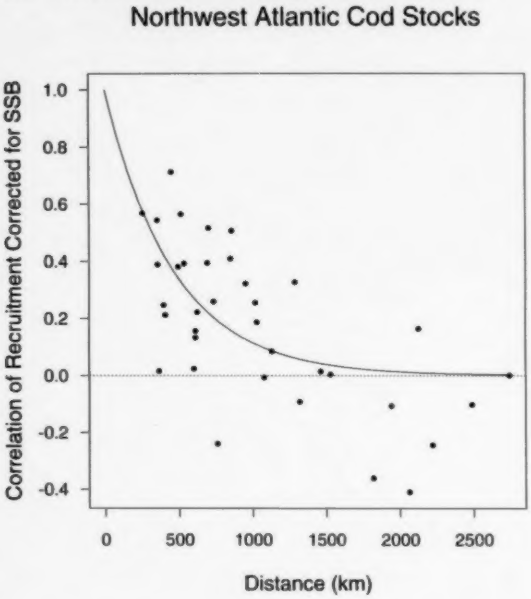


**Fig. 10.** Correlation of recruitment, estimated from the research surveys, versus distance for cod stocks in the Northwest Atlantic. The estimated decay parameter is $\hat{\theta} = 0.00134\ \mathrm{km}^{-1}$ (SE = 0.0003), i.e., an *e*-folding distance of 746 km.

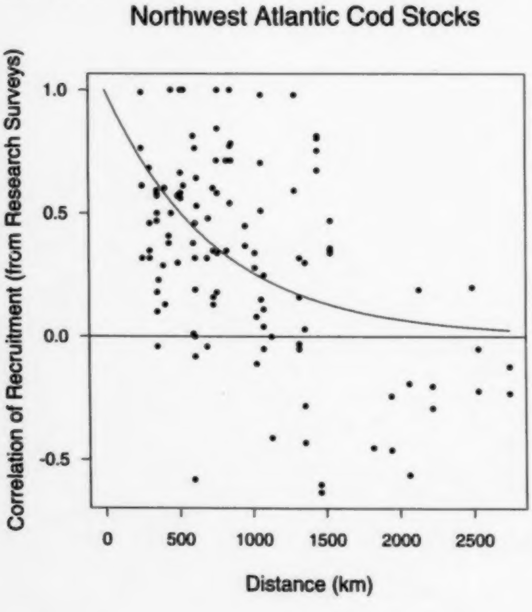


**Fig. 11.** Correlation of average yearly air temperature anomalies versus distance for maritime stations in the Northwest Atlantic from Godthaab, Greenland, to Cape Hatteras with over 50 years of data (Drinkwater 1994). The estimated decay parameter is $\hat{\theta} = 0.000686\ \mathrm{km}^{-1}$ (SE = 0.00005), i.e., an *e*-folding distance of 1458 km.

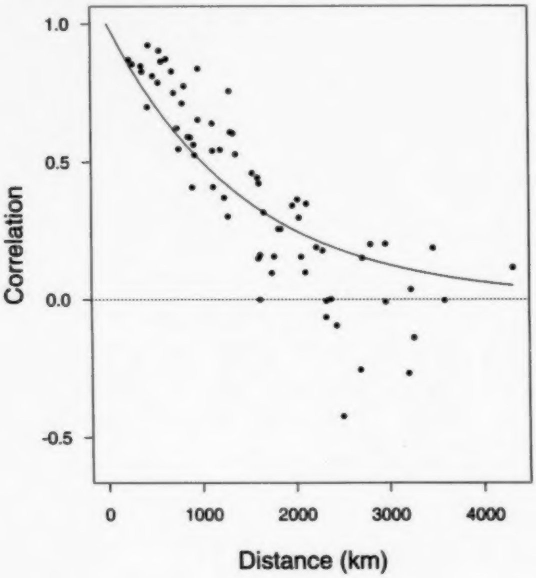


**Fig. 12.** Correlation of residuals from the smooth of recruitment, corrected for spawner biomass, from SPA versus distance for cod stocks in the Northeast Atlantic. The estimated decay parameter is $\hat{\theta} = 0.00296\ \mathrm{km}^{-1}$ (SE = 0.00047), i.e., an *e*-folding distance of 338 km.

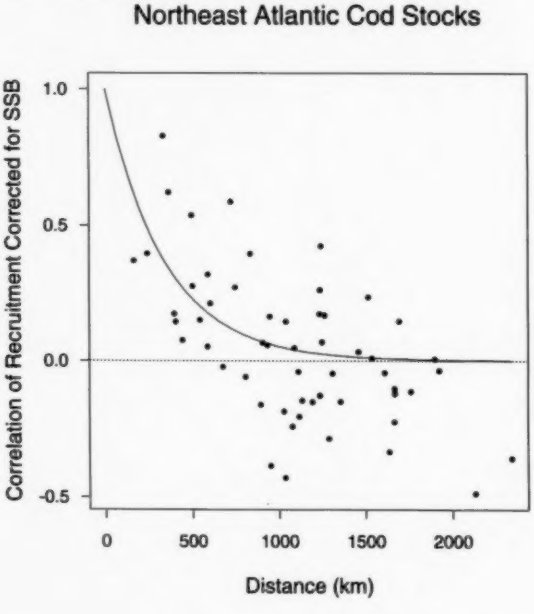


**Table 3.** Correlations and the associated *p* values between various regions and surveys produced by the PROC CALIS procedure.

| Region | 2J3KL spring | 3L spring | 3NO spring (Can) | 3NO spring (Rus) | 3Ps spring (Can) | 3Ps spring (Rus) | 3Pn4RS winter | 4TVn summer | 4VsW spring | 4WsV summer | 4X spring | 5Y spring (US) | 5Y fall (US) | 5Z spring (US) | 5Z fall (US) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VPA* | −63 | −60 | −19 | −56 | −46 | −24 | −45 | 19 | −20 | −29 | −20 | −22 | −5 | −23 | −12 |
|  | 0.071 | 0.085 | 0.44 | 0.10 | 0.31 | 0.43 | 0.13 | 0.39 | 0.60 | 0.35 | 0.54 | 0.36 | 0.84 | 0.33 | 0.61 |
| 2J3KL fall |  | 100+ | 46 | 76 | 66 | 56 | 64 | 28 | 100+ | 77 | 59 | 81 | 67 | 35 | 36 |
|  |  | 0.0067 | 0.18 | 0.055 | 0.19 | 0.11 | 0.051 | 0.34 | 0.029 | 0.043 | 0.097 | 0.024 | 0.065 | 0.24 | 0.24 |
| 3L spring |  |  | 32 | 19 | 96 | 58 | 53 | 34 | 100+ | 54 | 98 | 75 | 80 | 47 | 34 |
|  |  |  | 0.40 | 0.62 | 0.067 | 0.10 | 0.14 | 0.31 | 0.041 | 0.21 | 0.037 | 0.057 | 0.060 | 0.17 | 0.32 |
| 3NO spring (Can) |  |  |  | 100+ | 57 | −4 | 18 | −11 | −58 | 38 | 0 | −28 | −43 | −3 | −5 |
|  |  |  |  | 0.010 | 0.17 | 0.90 | 0.56 | 0.66 | 0.19 | 0.26 | 1.00 | 0.28 | 0.15 | 0.90 | 0.85 |
| 3NO spring (Rus) |  |  |  |  | 50 | 10 | 71 | 8 | 1 | 81 | −41 | 30 | 3 | 32 | 16 |
|  |  |  |  |  | 0.33 | 0.75 | 0.063 | 0.77 | 0.98 | 0.047 | 0.38 | 0.32 | 0.91 | 0.33 | 0.61 |
| 3Ps spring (Can) |  |  |  |  |  | 79 | 60 | 32 | 58 | 18 | 71 | 15 | 51 | 25 | 4 |
|  |  |  |  |  |  | 0.21 | 0.18 | 0.36 | 0.34 | 0.71 | 0.13 | 0.66 | 0.23 | 0.48 | 0.92 |
| 3Ps spring (Fr) |  |  |  |  |  |  | 13 | −4 | 59 | 47 | 78 | 98 | 70 | 11 | −5 |
|  |  |  |  |  |  |  | 0.65 | 0.88 | 0.17 | 0.17 | 0.099 | 0.026 | 0.063 | 0.72 | 0.85 |
| 3Pn4RS winter |  |  |  |  |  |  |  | 29 | 50 | 100+ | 48 | 35 | 71 | 45 | 37 |
|  |  |  |  |  |  |  |  | 0.34 | 0.24 | 0.019 | 0.16 | 0.25 | 0.050 | 0.12 | 0.23 |
| 4TVn summer |  |  |  |  |  |  |  |  | 30 | 57 | 23 | 38 | 41 | −8 | 0 |
|  |  |  |  |  |  |  |  |  | 0.46 | 0.061 | 0.39 | 0.13 | 0.12 | 0.72 | 0.99 |
| 4VsW spring |  |  |  |  |  |  |  |  |  | 100+ | 100+ | 84 | 100+ | 60 | 35 |
|  |  |  |  |  |  |  |  |  |  | 0.025 | 0.028 | 0.075 | 0.025 | 0.17 | 0.42 |
| 4VsW summer |  |  |  |  |  |  |  |  |  |  | 61 | 34 | 58 | 16 | 13 |
|  |  |  |  |  |  |  |  |  |  |  | 0.096 | 0.26 | 0.14 | 0.57 | 0.68 |
| 4X spring |  |  |  |  |  |  |  |  |  |  |  | 76 | 99 | 61 | 32 |
|  |  |  |  |  |  |  |  |  |  |  |  | 0.019 | 0.017 | 0.032 | 0.27 |
| 5Y spring (US) |  |  |  |  |  |  |  |  |  |  |  |  | 100+ | 46 | 35 |
|  |  |  |  |  |  |  |  |  |  |  |  |  | 0.0015 | 0.059 | 0.16 |
| 5Y fall (US) |  |  |  |  |  |  |  |  |  |  |  |  |  | 68 | 32 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0.013 | 0.22 |
| 5Z spring (US) |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 100+ |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0.0010 |

**Note**: The upper value is the correlation multiplied by 100 and the lower value is the associated *p* value. A + indicates that there were negative eigenvalues. Can, Canada; Fr, France; Rus, Russia; US, U.S.A.

*West Greenland.

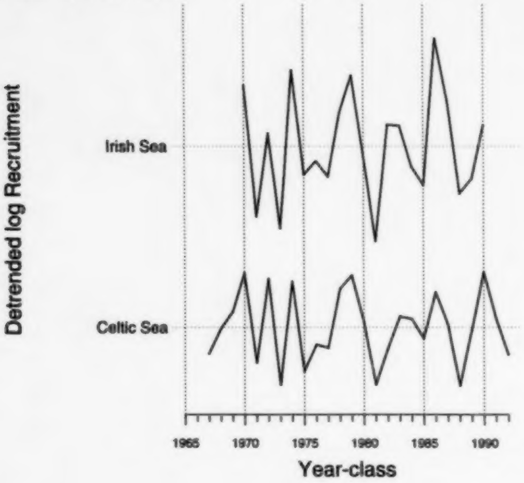
evident in both sets of data are artifacts. In both the research surveys and the SPA, the effect of most sources of bias will be to increase the correlations. For example, the research surveys carried out by the U.S.A. on Georges Bank and Gulf of Maine use a single ship, and changes in the survey characteristics would occur in both areas. Similarly, all of the Canadian surveys are performed using the same three or four ships, and changes in catchability associated with changes in a survey ship would affect simultaneously those surveys that use the same ship; this would produce positive bias in the correlations. For example, the Canadian survey in 3NO is highly correlated with the Canadian survey in 3Ps, much more so than French and Russian surveys. Since the two Canadian surveys were done using the same ships and crew, the high correlation may be spurious because of similar year-to-year biases and changes in methods.

Common factors other than environmental variations can affect the SPA estimates. For example, there may be common patterns in misreporting or discarding that would cause similar trends in the estimates. For example, fishing mortality increased greatly in the late 1980s for all Canadian cod populations at the same time population abundances declined (Myers et al. 1995*a*). This resulted in greater fishing mortality and discarding of small cod, which may have caused spurious declines in recruitment as estimated from SPA (Myers et al. 1995*c*). The simple detrending technique we have applied here seems to alleviate many of these problems, by eliminating the low frequency component of the data that is likely to be most contaminated by bias. The detrended series produce tenable spatial patterns of stock-pair correlations (Figs. 7*a*, 7*b*).

Some of the long-term trends in the SPA estimates of recruitment are probably real. Unfortunately we have no reliable way of separating the true trends from artifacts. We suggest that this part of the signal should be confirmed by analysis of research survey estimates of fish before they enter the fishery. It will be many decades before the reality of many of the long-term trends can be discerned from research surveys. There may be genuine long-period signals in the data that exhibit trans-Atlantic correlations, but we doubt it.

There have been many earlier analyses seeking spatial patterns in the SPA recruitment data for the North Atlantic. Although we have advanced this line of research the question remains as to how much information lies yet unrevealed

**Fig. 13.** Residuals of the recruitment, from SPA corrected for spawner biomass, from the long-term trend for the Celtic Sea and Irish Sea cod populations. Residuals are $\log_{10}$ transformed with the mean subtracted. The mean of each series is separated by 1 unit, i.e., a factor of 10, from the one below.



in the SPA data. In our opinion, there may be little to be learned from further searches for spatial patterns in the SPA data: Figs. 7*b* and 8 suggest that after the exponential decay with distance is removed, the residuals from the fit represent sampling variability alone. There is a suggestion of unanticipated genuine negative correlation of cod population from Iceland and West Greenland with a number of other populations (these correlations comprise only a very small proportion of the complete set). Although it is difficult ot assess the true statistical significance of these correlations, it is clearly worth testing them as more data accumulate on these populations.

Once the trends are removed from the SPA data, the decorrelation scale is comfortably less than the largest correlation scales found in the environmental data. Recruitment data that were correlated over larger distances than the environmental variables would be paradoxical.

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## Appendix

Consider a population, $p$, in which each cohort is surveyed several times. Let the number of fish in cohort $t$ surveyed at age $a$ be $\mathbf{N}_{p,t,a}$. The surveys see the juveniles at different ages; the survey index of the youngest age is 0, and survey indices with the fish at later ages are labeled sequentially. Let the average mortality between the first survey and the survey at age $a$, which is independent of density, be $m_{p,a}$. The population dynamics during the juvenile stage are described by

$$[3]\quad \mathbf{N}_{p,t,a} = \mathbf{N}_{p,t,0}e^{-m_{p,a}}$$

Here $m_{p,0} = 0$. Now consider the case of density-dependent mortality. We examine the hypothesis that mortality is linearly related to the logarithm of the initial number of juveniles in the cohort (Fig. 1). Let $(1 - \lambda_{p,a}) \log \mathbf{N}_{p,t,0}$ be the density-dependent mortality where $\lambda_{p,0} = 1$. We now have that

$$[4]\quad \mathbf{N}_{p,t,a} = \mathbf{N}_{p,t,0}e^{-m_{p,a}-(1-\lambda_{p,a}) \log \mathbf{N}_{p,t,0}}$$

Note that the density-dependent component of mortality is a function of the number of fish in the cohort at the first time they are seen in the surveys. It is useful to work with log-transformed variables; therefore, let $\mathbf{L}_{p,t,a} = \log \mathbf{N}_{p,t,a}$. Thus, the above equation becomes

$$[5]\quad \mathbf{L}_{p,t,a} = \lambda_{p,a}\mathbf{L}_{p,t,0} - m_{p,a}$$

Let $l_{p,t,a}$ be the log-transformed survey data. Estimates of abundance are assumed to be proportional to the true abundance. Let $q_{p,a}$ be the log catchability on age $a$ and let $\delta_{p,t,a}$ be the observational error on age $a$ of cohort $t$. The observed log-transformed abundance from age $a$ is

$$[6]\quad \begin{aligned} l_{p,t,a} &= q_{p,a} + \mathbf{L}_{p,t,a} + \delta_{p,t,a} \\ &= q_{p,a} - m_{p,a} + \lambda_{p,a}\mathbf{L}_{p,t,0} + \delta_{p,t,a} \end{aligned}$$

When estimating the parameters of eq. 6, the following assumptions are made about the stochastic nature of recruitment and estimation errors.

1. Relative year-class strength is determined in the larval or early juvenile stage, and there is no interannual variation in mortality in the juvenile stage unrelated to density. This assumption is tested in Myers and Cadigan (1993*b*).

2. Log-transformed standardized recruitments are mutually independent identically distributed random variables (i.e., independent of year) following a normal distribution with constant mean and variance. That is,

$$\mathbf{L}_{p,t,0} \sim N(\mu_p, \phi_p)$$

3. Errors in estimating abundance from research surveys, $\delta_{p,t,a}$, are mutually independent identically distributed random variables following a normal distribution with zero mean and constant variance. That is

$$\delta_{p,t,a} \sim N(0, \theta_{p,a})$$

Furthermore, the errors are independent of $\mathbf{L}_{p,t,0}$. The variance is constant in that it does not depend upon year, but it does depend upon age and survey.

Note that $q_{p,a} - m_{p,a}$ is constant for each survey and age. We do not attempt to estimate these terms, but merely subtract the sample mean over time for each survey and age from the data.

If possible we will use ages past the point that density-dependent mortality is important. That is, we attempt to avoid estimating the density-dependent mortality and assume that $\lambda_a = 1$. We will test the robustness of our conclusions to violations of this assumption.

Now consider the correlation in recruitment between two populations. Let the covariance in recruitment between populations $p$ and $q$ be

$$Cov(\mathbf{L}_{p,t,0}, \mathbf{L}_{q,t,0}) = \beta$$

The estimate of the correlation between the two populations is then

$$[7] \quad r = \frac{\hat{\beta}}{(\hat{\phi}_p \hat{\phi}_q)^{\frac{1}{2}}}$$

where estimates of the above covariances and variances are used. The correlations we estimate from the research surveys do not necessarily lie between −1 and 1, because they are derived parameters (eq. 7). That is, the estimates are sometimes greater than one because $\beta$ and the $\phi$'s are estimated with error. The correlation of recruitment between populations is assumed to interact through increased mortality that occurs before the first juvenile survey (eq. 4). Myers and Cadigan (1993*a*, 1993*b*) found that most cod populations had negative autocorrelations between adjacent year-classes. Therefore, we do not correct for autocorrelation in the research survey estimates.

With the above assumptions it is possible to estimate the unknown parameters in the models. This is done by using maximum likelihood methods to obtain the theoretical covariance matrix best fitting the observed covariance matrix of the research surveys. To illustrate this idea, we illustrate a simple case that can readily be explained, i.e., two populations, *p* and *q*, which are surveyed at ages 1 and 2 and for which there is no density-dependent mortality. The theoretical variance–covariance matrix, $\Sigma$, is

$$\begin{bmatrix} Var(l_{p,t,1}) & Cov(l_{p,t,1}, l_{p,t,2}) & Cov(l_{p,t,1}, l_{q,t,1}) & Cov(l_{p,t,1}, l_{q,t,2}) \\ & Var(l_{p,t,2}) & Cov(l_{p,t,2}, l_{q,t,1}) & Cov(l_{p,t,2}, l_{q,t,2}) \\ & & Var(l_{q,t,1}) & Cov(l_{q,t,1}, l_{q,t,2}) \\ & & & Var(l_{q,t,2}) \end{bmatrix} = \begin{bmatrix} \phi_p + \theta_{p,1} & \phi_p & \beta & \beta \\ & \phi_p + \theta_{p,2} & \beta & \beta \\ & & \phi_q + \theta_{q,1} & \phi_q \\ & & & \phi_q + \theta_{q,2} \end{bmatrix}$$

Note that the above matrix has seven unknown parameters to estimate and there are 10 terms in the upper triangular portion of the matrix. We estimate the other parameters in $\Sigma$ by matching the observed variance covariance matrix, **S**, as closely as possible. By inspection we see that each model parameter can be uniquely written as a function of one or more elements in **S**; that is, the model is identified. We use maximum likelihood methods to obtain the estimates. Details of the estimation methods are described in Bollen (1989). The basic estimation procedure is to fit the observed variance–covariance matrix with the theoretical one. The fitting function that is minimized to find the maximum likelihood estimates is

$$[8] \quad \log |\Sigma| + tr(\mathbf{S}\Sigma^{-1}) - \log(|\mathbf{S}|) - d$$

where *tr* is the matrix trace and *d* is the dimension of **S**. The maximization of eq. 8 results in the theoretical variance–covariance matrix $\Sigma$ that is closest to the observed matrix **S**. The models are estimated using PROC CALIS of the SAS Institute (Cary, N.C.) with the pairwise covariance matrix. If this matrix is not positive definite, the ridge option in PROC CALIS is used to perturb the matrix so that it is positive definite, or the casewise covariance matrix is used. As a test of whether $\beta \neq 0$, the *p* values are presented. These *p* values take into account the reliability of the surveys, i.e., the estimation error. In Table 3 we display the results under the assumption of no density-dependent mortality. We repeated the analysis estimating density-dependent mortality, and the results were virtually identical.

# Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations

**Stewart K. McConnell, Patrick O'Reilly, Lorraine Hamilton, Jonathan M. Wright, and Paul Bentzen**

**Abstract**: Atlantic salmon populations show low levels of genetic differentiation relative to other salmonid species, when surveyed by allozymes, and with mitochondrial DNA and nuclear ribosomal DNA markers. Here we report the application of three novel microsatellite VNTR loci to population differentiation in Atlantic salmon. A total of 232 microsatellites, cloned from Atlantic salmon, were classified as perfect, imperfect, and compound repeats. Microsatellite length, as in other teleosts, was significantly larger than published mammalian microsatellites. Primers for PCR amplification of three salmon microsatellites were designed. Allele frequencies, degree of polymorphism, and heterozygosity were estimated for five populations from Nova Scotia, Canada, and from Europe. Nei's genetic distances of 0.02–0.9 were observed among populations. There was a clear discrimination between Canadian and European fish based on unique alleles present at two loci. These Atlantic salmon primers also amplify presumably homologous loci in nine other salmonid species. The polymorphic microsatellites loci reported here demonstrate great potential as genetic markers in population, breeding, and evolutionary studies.

**Résumé** : Les populations de saumon atlantique présentent de faibles niveaux de différenciation génétique par rapport à d'autres espèces de salmonidés, selon notre étude faisant appel aux allozymes et aux marqueurs de l'ADN mitochondrial et de l'ADN ribosomique nucléaire. Nous rapportons ici l'application à la différenciation des populations de saumon atlantique de trois nouveaux loci à nombre variable de séquences répétées en tandem correspondant à des microsatellites. Au total, 232 microsatellites, obtenus par clonage chez le saumon atlantique, ont été classés comme séquences répétées parfaites, imparfaites et composées. La longueur des microsatellites, comme chez d'autres téléostéens, était nettement plus grande que celle des microsatellites de mammifères décrits dans la littérature. Nous avons conçu des amorces pour l'amplification génique des microsatellites de trois saumons. Nous avons estimé la fréquence des allèles, le degré de polymorphisme et l'hétérozygotie pour cinq populations de Nouvelle-Écosse, Canada, et d'Europe. Nous avons observé des distances génétiques de Nei de 0,02–0,9 entre les populations. Il y avait une nette discrimination entre les poissons canadiens et européens, qui se fondait sur des allèles particuliers présents sur deux loci. Ces amorces chez le saumon atlantique amplifient aussi des loci probablement homologues chez neuf autres espèces de salmonidés. Les loci des microsatellites polymorphes présentés ici offrent un grand potentiel comme marqueurs génétiques dans les études sur les populations, la reproduction, et l'évolution.

[Traduit par la Rédaction]

## Introduction

Genetic variation in salmonid populations has been extensively studied, using a wide range of genetic markers. Genetic studies of Atlantic salmon using allozymes, mitochondrial DNA markers, and nuclear ribosomal DNA markers have revealed genetic variation in some cases (e.g., Bermingham et al. 1991; Birt et al. 1991; Cutler et al. 1991) but have generally detected only low levels of genetic variation (Davidson et al. 1989; McElligott and Cross 1991).

The discovery of high levels of polymorphism at minisatellite (Jeffreys et al. 1985) or VNTR (variable number

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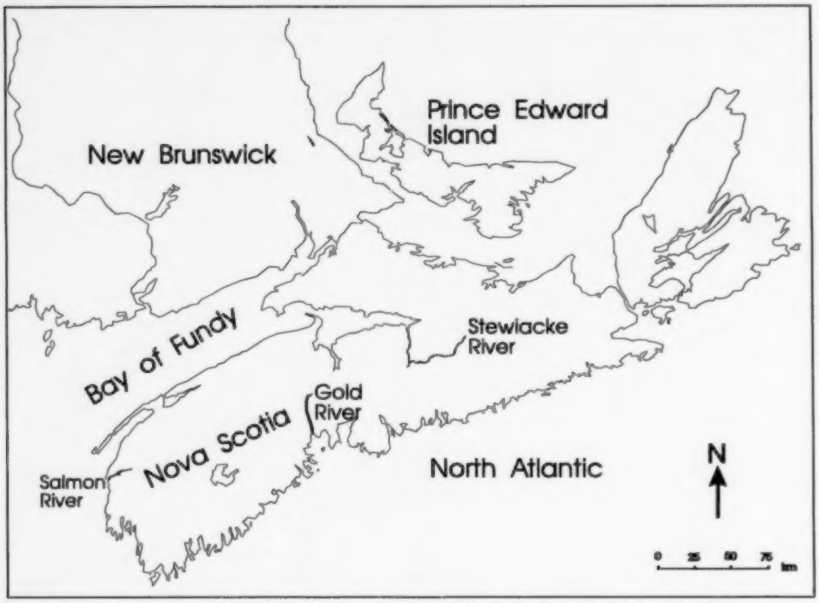
**Fig. 1.** A map of the Atlantic provinces of Canada showing the location of the three Nova Scotian rivers from which Atlantic salmon were surveyed for genetic variation at three microsatellite loci.



of tandem repeat) loci (Nakamura et al. 1987) has provided a powerful tool for studies of genetic variation among populations. Initial studies focused on the use of minisatellite loci for population differentiation (Baird et al. 1986; Balazs et al. 1989; Flint et al. 1989; Gilbert et al. 1990; Bentzen et al. 1991; Bruford and Burke 1991); however, more recently, microsatellite markers have been developed in a range of species (e.g., Weber 1990; Ellegren et al. 1992; Love et al. 1990; Goff et al. 1992; Brooker et al. 1994). Microsatellites consist of tandemly arrayed di-, tri-, or tetranucleotide repeat sequences, flanked by regions of nonrepetitive DNA (Tautz 1989; Weber and May 1989; Litt and Luty 1989). Alleles at microsatellite loci can be amplified by the polymerase chain reaction (PCR) (Saiki et al. 1988) from small samples of genomic DNA, and the products can be size fractionated on a polyacrylamide gel.

Surveys of the genetic variation present in salmonid populations using multi-locus and single-locus VNTR probes (Taggart and Ferguson 1991; Bentzen et al. 1991; Bentzen et al. 1993; Bentzen et al. 1995; Prodöhl et al. 1994) have yielded promising results showing resolution at the level of individual, subpopulation, and population (Taggart and Ferguson 1990; Taylor et al. 1993, 1994).

The application of polymorphic microsatellite loci to questions of population genetics and ecology is still in its infancy. While the main thrust of microsatellite marker development has been for genome mapping (Rohrer et al. 1994; Dietrich et al. 1992; Ostrander et al. 1993), a number of studies have applied microsatellites to population genetics problems (Edwards et al. 1992; Bowcock et al. 1994; Brooker et al. 1994; Roy et al. 1994).

In this study we report the isolation of microsatellite loci from the Atlantic salmon genome. Primers were designed for three loci and their amplification conditions were optimized. Levels of variation at these loci and the ability to discriminate between five populations of Atlantic salmon from Europe and North America were determined. Primers designed to amplify microsatellite loci in Atlantic salmon also amplified presumably homologous loci in related species.

## Materials and methods

### Samples

Population polymorphisms at microsatellite loci were examined in three Atlantic salmon (*Salmo salar*) populations from eastern Canada and three European populations. Parr from three Nova Scotian populations, Gold River ($n = 35$), Salmon River ($n = 20$), and Stewiacke River ($n = 45$), were collected by electrofishing (courtesy of P. Steele, Department of Fisheries and Oceans, Canada; Fig. 1). DNA from tissue samples of returning spawners from two rivers in southwest Norway, the Dale and Onarheim ($n = 10$ per population), were provided by G. Dahle (Institute of Marine Research, Bergen, Norway). Samples from the River Corrib, County Galway, Ireland, ($n = 26$) were provided by J. Taggart (University of Stirling, U.K.). Allelic segregation for the microsatellite loci was analyzed in two full-sib families from the Atlantic Salmon Federation hatchery, Passamaquoddy Bay, St. Andrews, N.B. (10 progeny per family).

DNA extraction in all samples, except for the full-sib families, was from muscle tissue using the modified

**Table 1.** Microsatellite sequences, PCR primers, and locus-specific annealing temperatures for three Atlantic salmon (*Salmo salar*) microsatellite loci, *Ssa4*, *Ssa14*, and *Ssa289*.

| Locus | Microsatellite sequence | Primer | Temperature (°C) |
|---|---|---|---|
| *Ssa4* | $(GT)_{39}$ | 5′-ATTAGGCAGCAGCAGGCTGC<br>5′-TGTTCACTCACTGACACGCG | 65 |
| *Ssa14* | $(TC)_{10}N_{15}(TC)_3N_2(AC)_{12}(TC)_3N_5(CA)_4$ | 5′-CCTTTTGACAGATTTAGGATTTC<br>5′-CAAACCAAACATACCTAAAGCC | 57 |
| *Ssa289* | $(GT)_{12}$ | 5′-CTTTACAAATAGACAGACT<br>5′-TCATACAGTCACTATCATC | 46 |

**Table 2.** Microsatellite sequences isolated from Atlantic salmon and categorized according to Weber (1990).

| | Atlantic salmon (this study) | | Atlantic salmon[a] | | Trout[b] | | Cod[c] | |
|---|---|---|---|---|---|---|---|---|
| Category | *n* | % | *n* | % | *n* | % | *n* | % |
| Perfect | 164 | 71 | 36 | 80 | 29 | 57 | 31 | 48 |
| Imperfect | 53 | 23 | 9 | 20 | 16 | 31 | 30 | 45 |
| Compound | 15 | 6 | 0 | 0 | 6 | 12 | 4 | 7 |

**Note**: Microsatellite surveys from three other teleosts are shown for comparison.

[a] Slettan et al. 1993.

[b] Morris et al. 1996.

[c] Brooker et al. 1994.

phenol–chloroform protocol of Taggart et al. (1992). Full-sib family DNA was extracted from blood samples using a salt extraction method (Millar et al. 1988).

### Cloning and sequencing of microsatellites

Three partial genomic DNA libraries were constructed from Atlantic salmon DNA. For the first library, DNA samples from two Irish and two Nova Scotian individuals were pooled. Aliquots of 35 μg of pooled DNA were digested to completion with 100 units of one of the enzymes *Mbo*I, *Pal*I, *Rsa*I, and *Alu*I. Digested samples were size fractionated in a 1.5% agarose gel, the 200–500 base pair (bp) fraction was excised, and the DNA purified using a phenol– chloroform extraction procedure. *Mbo*I-digested fragments were ligated into the *Bam*HI site of dephosphorylated pUCBM20 vector. Fragments from the other digests were ligated into the *Sma*I site of pUC18. For the second library, 50 μg of DNA from one Nova Scotian salmon was digested with four enzymes combined in one reaction (*Rsa*I, *Hinc*II, *Pal*I, *Alu*I: 100 units of each) in an attempt to increase the proportion of fragments within the size-selected range. A 300–600 bp fraction was isolated and ligated into the *Sma*I site of pUC18. For the third library, 50 μg of DNA from one Nova Scotian salmon was digested with *Sau*3A (100 units) and ligated into the dephosphorylated *Bam*HI site of pUC18. Plasmids from all ligation reactions were transformed into Maximum Efficency DH5α competent cells (Gibco BRL) according to the manufacturer's protocol. Colony blots were then carried out onto nylon membrane filters following the manufacturer's protocol (Hybond N, Amersham).

Libraries were screened for microsatellite repeats using a $(GT)_{15}$ oligonucleotide repeat 5′-end labelled with [$\gamma$-$^{32}$P]ATP using a standard protocol (Pharmacia). Colony lifts were hybridized to the oligonucleotide $(GT)_{15}$ overnight at 62°C using the reaction conditions of Westneat et al. (1988). Washes were 2× SSC, 0.2% SDS at room temperature and 0.2× SSC, 0.2% SDS at 62°C.

Alkaline-denatured plasmid template DNA was sequenced by the dideoxynucleotide chain termination method (Sanger et al. 1977) with a T7 sequencing kit (Pharmacia). PCR primers were designed to the unique regions flanking the core repeat sequences for three microsatellite sequences, *S. salar* clone 4 (*Ssa4*), *S. salar* clone 14 (*Ssa14*), and *S. salar* clone 289 (*Ssa289*). Loci *Ssa4* and *Ssa14* were isolated from genomic library 1 and locus *Ssa289* was isolated from library 2. These three clones were initially chosen for further characterization as a result of the large amount of flanking sequence available for primer design.

### Microsatellite polymorphisms

Microsatellite loci were amplified by the polymerase chain reaction (PCR) to assess the level of population polymorphism. Amplification was performed in a PTC-100 Thermal Cycler (M.J. Research Inc.) in a 10 μL reaction volume.

**Fig. 2.** Number of dinucleotide repeat units, taken as the number of repeats within the longest run of uninterrupted repeats, versus number of sequences for microsatellites isolated from Atlantic salmon (*Salmo salar*).

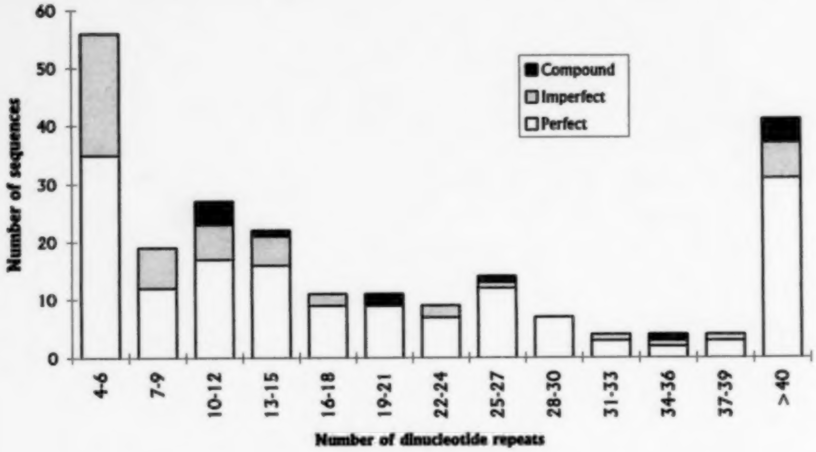
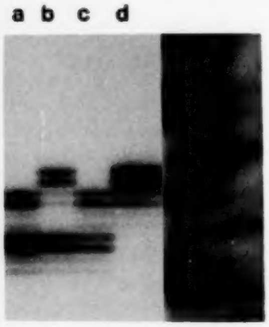
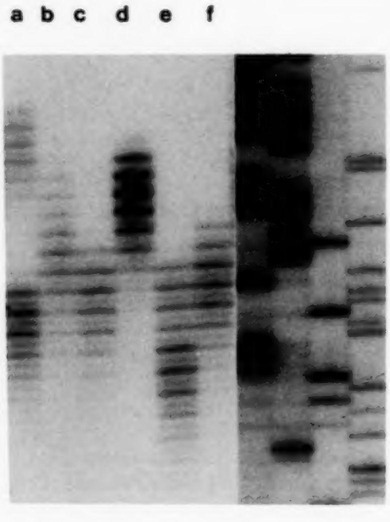


**Fig. 3.** PCR products for locus *Ssa289* from four individuals. An M13 sequence ladder is shown on the right. Individual genotypes in base pairs are as follows: *a* (113/117), *b* (113/119), *c* (113/117), and *d* (119/119).



This consisted of 10–40 ng of genomic DNA, 0.6 μM primer 1, 0.56 μM primer 2, 200 μM dNTP's (50 μM each of dGTP, dCTP, dTTP, and dATP), and 0.25 units of Taq polymerase in 1× reaction buffer (10× buffer is 100 mM Tris–HCl, ph 8.3, 10 mM $MgCl_2$, 500 mM KCl, 0.1% gelatin). The product was labelled by the inclusion of 0.04 μM of primer 2, 5′-end labelled with [$\gamma$-$^{32}$P]ATP. Samples were amplified for 35 cycles consisting of 30 s at 94°C, 30 s at the primer-specific temperature (see Table 1), and 30 s at 72°C. An equal volume of formamide loading buffer was then added and the product was denatured for 15 min at 95°C before being subjected to electrophoresis on a standard DNA sequencing gel (5 M urea, 6–8% acrylamide). Gels were fixed, dried, and exposed to autoradiographic film at −80°C for 4–16 h. A standard M13 sequencing reaction was used as a size marker. On each gel, standard samples of known size were loaded to enable consistent scoring of allele sizes between gels.

**Fig. 4.** PCR products for locus *Ssa4* from six individuals. An M13 sequencing ladder is shown on the right. Individual genotypes in base pairs are as follows: *a* (116/136), *b* (122/130), *c* (116/120), *d* (130/130), *e* (110/118), and *f* (118/122).



For each population, at each locus, the number of alleles present and observed heterozygosity were calculated. Genotype frequencies in each population at each locus were tested for conformity to Hardy–Weinberg equilibrium using the CHIHW program in the RANDOM package (Zaykin and Pudovkin 1993). The program tests the significance of the $\chi^2$ value for the original sample to $\chi^2$ values for random samples of the data. The result of the Monte Carlo simulation is the proportion of the number of resampled data sets for which the $\chi^2$ is greater than or equal to the $\chi^2$ for the

**Table 3.** Observed allele number and allele size range for three microsatellite loci in five Atlantic salmon populations from Nova Scotia, Norway, and Ireland.

| Locus | Gold | Salmon | Stewiacke | Norwegian | Irish |
|---|---|---|---|---|---|
| *Ssa4* | | | | | |
| No. alleles | 11 | 8 | 11 | 16 | 13 |
| Size range | 114–144 | 120–156 | 114–144 | 114–190 | 112–192 |
| $H_{obs}$[a] | 0.89 | 0.6 | 0.65 | 0.65 | 0.69 |
| *Ssa14* | | | | | |
| No. alleles | 2 | 2 | 3 | 3 | 3 |
| Size range | 141–145 | 141–145 | 133–145 | 141–145 | 141–145 |
| $H_{obs}$ | 0.36 | 0.35 | 0.56 | 0.35 | 0.46 |
| *Ssa289* | | | | | |
| No. alleles | 4 | 2 | 3 | 6 | 3 |
| Size range | 113–119 | 113–119 | 113–119 | 113–125 | 117–121 |
| $H_{obs}$ | 0.38 | 0.3 | 0.59 | 0.75 | 0.44 |

**Note**: Two Norwegian populations ($n$ = 10 per population) are pooled. Allele size is in base pairs.

[a] $H_{obs}$, observed heterozygosity.

original table. This proportion is taken as the probability of the null hypothesis (conforming to Hardy–Weinberg; *P* (null hypothesis)). Tests of heterogeneity between populations were performed using a Monte Carlo analogue of the $\chi^2$ test (Roff and Bentzen 1989) as implemented in the CHRXC program in the RANDOM package. Results are expressed as the probability of homogeneity between or among samples (*P* (homogeneity)). Allele frequencies were then used to calculate Nei's Unbiased Genetic Distance (Nei 1978) using NTSYS-pc version 1.8 (Rohlf 1993).

## Results

A total of 232 microsatellites (194 $(GT)_n$, 8 $(CT)_n$, 5 $(AT)_n$, 10 $(GA)_n$, and 15 compound repeats) were isolated from three size-selected libraries of Atlantic salmon genomic DNA. The percentages of positive clones in libraries 1 (23/2000) and 3 (178/10 400) were 1.15 and 1.71%, respectively. (An estimate for the abundance of $(GT)_n$ tracts in library 2 was not made.) Assuming that (*i*) the fraction of the salmon genome cloned in each library was a random representative sample of the total genome; (*ii*) that $(GT)_n$ tracts were randomly distributed within this faction; (*iii*) an average insert size for each clone was 400 bp; (*iv*) a haploid genome size was $6 \times 10^9$ bp (Hamada et al. 1982), we estimate that $1.7–2.5 \times 10^5$ $(GT)_n$ loci are present in the Atlantic salmon genome and occur every 24–35 kb.

The microsatellite sequences isolated were categorized according to Weber (1990) as perfect, imperfect, and compound (Table 2). Perfect sequences made up 71% of those isolated. A significant proportion of sequences (18%) were 40 or more repeat units in length, with the largest being an imperfect $(GT)_n$ repeat of 82 dinucleotide units (see Fig. 2).

### Population polymorphism

Allelic segregation at three microsatellite loci, *Ssa4*, *Ssa14*, and *Ssa289* (Table 1; Figs. 3 and 4) was examined in two full-sib families. Genotype frequencies observed at all loci were in agreement with expected Mendelian proportions. When tested with *Ssa4*, however, one member of a progeny group contained an allele not present in either parent. This fish may have been misclassified as a progeny group member as a result of "jumping" between progeny group holding tanks. An alternative explanation is that there has been a mutation at this locus. Screening at other microsatellite loci is necessary to ascertain the probability of this fish being a progeny group member.

**Fig. 5.** UPGMA dendrogram of Nei's (1978) unbiased genetic distance for five populations of Atlantic salmon including three populations from Nova Scotia, Canada (Gold, Salmon, and Stewiacke rivers), and two populations from Europe (Norway and Ireland). The Norwegian sample is comprised of fish from two river populations ($n$ = 10 per population).

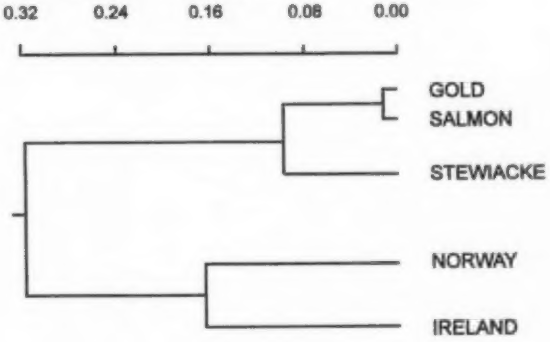


Table 3 lists numbers of alleles, size range of alleles, and observed heterozygosities at the microsatellite loci for the three Nova Scotian populations (see Fig. 1), a pooled sample of the two Norwegian populations and the one Irish population. All populations conformed to Hardy–Weinberg expectations. The two Norwegian samples were not significantly different at any of the microsatellite loci and thus were treated as one pooled sample. Allele frequencies at the loci *Ssa4*, *Ssa14*, and *Ssa289* for all samples are listed in Table 4. Highly significant differences in allele

**Table 4.** Allele frequencies observed at three microsatellite loci in Atlantic salmon populations from Nova Scotia, Norway, and Ireland.

| Allele | Gold (*n* = 35) | Salmon (*n* = 20) | Stewiacke (*n* = 45) | Norwegian (*n* = 20) | Irish (*n* = 26) |
|---|---|---|---|---|---|
| | | | ***Ssa4*** | | |
| *112* | — | — | — | — | 0.30 |
| *114* | 0.07 | — | 0.06 | 0.02 | — |
| *116* | 0.02 | — | 0.02 | 0.10 | 0.02 |
| *118* | 0.04 | — | 0.17 | 0.02 | 0.15 |
| *120* | 0.04 | 0.06 | — | 0.13 | 0.11 |
| *122* | — | — | 0.05 | — | 0.06 |
| *124* | 0.02 | — | 0.04 | — | — |
| *126* | 0.12 | 0.11 | 0.05 | — | — |
| *128* | 0.15 | 0.09 | 0.10 | — | — |
| *130* | 0.25 | 0.32 | 0.40 | — | — |
| *132* | — | 0.09 | — | — | — |
| *134* | 0.03 | 0.03 | 0.02 | — | — |
| *136* | 0.04 | 0.09 | — | — | — |
| *138* | — | — | — | 0.08 | — |
| *140* | 0.10 | 0.15 | 0.08 | — | 0.02 |
| *142* | 0.06 | 0.03 | — | — | — |
| *144* | 0.06 | — | 0.01 | — | — |
| *146* | — | — | — | — | — |
| *148* | — | 0.03 | — | 0.05 | — |
| *150* | — | — | — | 0.33 | 0.09 |
| *152* | — | — | — | 0.03 | 0.02 |
| *156* | — | — | — | — | — |
| *158* | — | — | — | — | — |
| *160* | — | — | — | 0.05 | — |
| *162* | — | — | — | 0.02 | — |
| *168* | — | — | — | 0.02 | — |
| *170* | — | — | — | 0.01 | — |
| *178* | — | — | — | — | 0.02 |
| *180* | — | — | — | — | 0.04 |
| *182* | — | — | — | — | 0.04 |
| *184* | — | — | — | 0.03 | — |
| *186* | — | — | — | 0.03 | — |
| *188* | — | — | — | 0.05 | 0.02 |
| *190* | — | — | — | 0.02 | 0.09 |
| | | | ***Ssa14*** | | |
| *133* | — | — | 0.04 | — | — |
| *141* | 0.70 | 0.74 | 0.43 | 0.63 | 0.42 |
| *143* | — | — | — | 0.02 | 0.06 |
| *145* | 0.29 | 0.26 | 0.52 | 0.35 | 0.52 |
| | | | ***Ssa289*** | | |
| *113* | 0.81 | 0.85 | 0.50 | 0.08 | — |
| *115* | 0.05 | — | — | — | — |
| *117* | 0.03 | — | 0.04 | 0.08 | 0.52 |
| *119* | 0.10 | 0.15 | 0.46 | 0.40 | 0.44 |
| *121* | — | — | — | 0.20 | 0.04 |
| *123* | — | — | — | 0.22 | — |
| *125* | — | — | — | 0.02 | — |

**Note**: The Norwegian sample is composed of two populations (*n* = 10 per population).

frequencies were observed in pairwise comparisons between samples using the Bonferroni procedure (Rice 1989), when performed separately at each locus (Table 5). Allele frequencies at the three loci were used to calculate Nei's (1978) Unbiased Genetic Distance between samples (Table 6) and a UPGMA dendrogram was constructed (Fig. 5).

When genetic distances were calculated individually for each locus, *Ssa4* and *Ssa289* both showed large distance values for sample comparisons between Europe and North America ($D = 0.38–3.23$). At locus *Ssa4* in Nova Scotian populations, allele sizes ranged from 114 to 148 bp, while in the two European populations allele sizes ranged from 112 to 190 bp. A large proportion of "European" alleles were in the size range 150–190 bp. Alleles *Ssa4*$^{124}$, *Ssa4*$^{126}$, *Ssa4*$^{128}$, *Ssa4*$^{130}$, and *Ssa4*$^{132}$, which were present at cumulative frequencies of 0.54–0.61 within Nova Scotian populations, are completely absent in the European samples. Within continents, *Ssa4* also shows highly significant differences between populations. In Nova Scotia, only the $\chi^2$ contingency test between the Gold and Salmon rivers was not significant (Table 5). The Irish sample contains an allele *Ssa4*$^{112}$ that is absent from the Norwegian sample. In 20 other fish from two Norwegian rivers, *Ssa4*$^{112}$ was not observed (data not shown).

At locus *Ssa289* three alleles were observed in the European populations that were not present in the Nova Scotian samples (*Ssa289*$^{121}$, *Ssa289*$^{123}$, *Ssa289*$^{125}$, Table 4). All $\chi^2$ contingency test comparisons between populations were significant except for the Gold and Salmon river comparison (Table 5).

Locus *Ssa14*, the least polymorphic locus, showed no difference between European and Nova Scotian samples. Within Nova Scotia, however, a significant difference in allele frequencies was observed between the Stewiacke River on the Bay of Fundy and the Gold and Salmon rivers on the Atlantic shore ($P = 0.001$, $P = 0.002$, respectively; Table 5).

### Amplification of microsatellite loci in other salmonine species

Primers for PCR amplification of the Atlantic salmon microsatellite loci, *Ssa4*, *Ssa14*, and *Ssa289*, were tested for their ability to amplify presumably homologous microsatellite loci in nine other salmonine species (Table 7). Products were observed in all cases except with *Ssa4* in pink salmon and with *Ssa289* in sockeye, coho, chum, and pink salmon. The PCR conditions used were those optimal for amplification of microsatellite loci from Atlantic salmon and thus may not be optimal in all the other salmonine species. Locus *Ssa14* showed a low level of variation in all the species tested, while locus *Ssa4* showed high levels of variation in most species with up to nine alleles present in a sample of six individuals. The degree of relatedness between sample individuals was unknown, and in some species where the number of alleles observed was low, this may be due to samples coming from closely related fish. In all the salmonines tested, the size range of alleles observed was similar to that observed in Atlantic salmon. Locus *Ssa289* showed levels of variation in other salmonids similar to those observed in Atlantic salmon; however, in brown trout, the locus appeared to be tetraploid with three to four alleles per individual. The allele size

**Table 5.** Pairwise comparisons of allele frequencies between five Atlantic salmon populations at microsatellite loci *Ssa4*, *Ssa14*, and *Ssa289*.

| | Population | | | | |
|---|---|---|---|---|---|
| **Population** | **1** | **2** | **3** | **4** | **5** |
| ***Ssa4*** | | | | | |
| 1 | — | ns<br>$P = 0.19$ | **<br>$P = 0.003$ | ****<br>$P = 0$ | ****<br>$P = 0$ |
| 2 | | — | ****<br>$P = 0$ | ****<br>$P = 0$ | ****<br>$P = 0$ |
| 3 | | | — | ****<br>$P = 0$ | ****<br>$P = 0$ |
| 4 | | | | — | ****<br>$P = 0$ |
| ***Ssa14*** | | | | | |
| 1 | — | ns<br>$P = 0.67$ | ****<br>$P = 0.001$ | ns<br>$P = 0.37$ | ****<br>$P = 0.002$ |
| 2 | | — | ****<br>$P = 0.002$ | ns<br>$P = 0.32$ | ****<br>$P = 0.003$ |
| 3 | | | — | *<br>$P = 0.032$ | ns<br>$P = 0.054$ |
| 4 | | | | — | ns<br>$P = 0.144$ |
| ***Ssa289*** | | | | | |
| 1 | — | ns<br>$(P = 0.3)$ | ****<br>$(P = 0)$ | ****<br>$(P = 0)$ | ****<br>$(P = 0)$ |
| 2 | | — | ****<br>$(P = 0)$ | ****<br>$(P = 0)$ | ****<br>$(P = 0)$ |
| 3 | | | — | ****<br>$(P = 0)$ | ****<br>$(P = 0)$ |
| 4 | | | | — | ****<br>$(P = 0)$ |

**Note:** For each pairwise comparison the significance of the $\chi^2$ value and the probability of sample homogeneity ($P$) (Zaykin and Pudovkin 1993) are shown. 1, Gold; 2, Salmon; 3, Stewiacke; 4, Norway; 5, Ireland.

*, $P < 0.05$; **, $P < 0.025$; ****, $P < 0.005$; ns, not significant.

ranges observed in brown and brook trout, chinook salmon, and Arctic char were larger than those in Atlantic salmon (Table 7).

## Discussion

Dinucleotide microsatellite repeat sequences appear to be abundant in the Atlantic salmon genome. Previous estimates of $(\mathrm{GT})_n$ repeat sequence abundance in salmon range from every 90 kb and the presence of $6.3 \times 10^4$ loci (Slettan et al. 1993) to $2 \times 10^5$ loci (Hamada et al. 1982). In brown trout, Estoup et al. (1993) estimate $(\mathrm{GT})_n$ to occur every 23 kb, with $1.9 \times 10^5$ loci present. Our estimates of $1.7–2.5 \times 10^5$ $(\mathrm{GT})_n$ loci in Atlantic salmon are considerably higher than those of Slettan et al. (1993), but are in agreement with estimates in brown trout by Estoup et al. (1993).

The higher frequency of large microsatellite sequences observed in teleosts as compared with mammals has been

**Table 6.** Nei's (1978) unbiased genetic distances among five Atlantic salmon populations from Nova Scotia, Norway, and Ireland, at microsatellite loci, *Ssa4*, *Ssa14*, and *Ssa289*.

| | Population | | | | |
|---|---|---|---|---|---|
| **Population** | **1** | **2** | **3** | **4** | **5** |
| 1. Gold | — | 0.02 | 0.18 | 0.54 | 0.85 |
| 2. Salmon | | — | 0.18 | 0.55 | 0.90 |
| 3. Stewiacke | | | — | 0.40 | 0.45 |
| 4. Norway[a] | | | | — | 0.32 |
| 5. Ireland[b] | | | | | — |

[a] Pooled sample of two populations ($n = 10$ per population) from the Dale and Onarheim rivers.

[b] Corrib River, County Galway.

**Table 7.** PCR amplification of microsatellite loci in salmonids using Atlantic salmon derived primers for *Ssa4*, *Ssa14*, and *Ssa289*.

| | *Ssa4* | | *Ssa14* | | *Ssa289* | |
|---|---|---|---|---|---|---|
| Species | No. of alleles | Size range | No. of alleles | Size range | No. of alleles | Size range |
| Genus *Salmo* | | | | | | |
| Atlantic salmon | 34 | 112–190 | 4 | 133–145 | 7 | 113–125 |
| Brown trout | 1 | 120 | 2 | 132–134 | 14[a] | 113–233 |
| Genus *Onchorynchus* | | | | | | |
| Chinook | 7 | 142–162 | 3 | 163–169 | 7 | 178–222 |
| Sockeye | 7 | 126–190 | 1 | 139 | 0 | |
| Coho | 2 | 112–118 | 2 | 146–148 | 0 | |
| Chum | 4 | 128–232 | 3 | 116–132 | 0 | |
| Pink | 0 | | 1 | 125 | 0 | |
| Rainbow | 9 | 171–223 | 3 | 132–142 | 4 | 105–117 |
| Genus *Salvelinus* | | | | | | |
| Brook trout | 2 | 96–120 | 1 | 146 | 2 | 179–231 |
| Arctic char | | — | | — | 3 | 188–196 |

**Note**: Six individuals were examined per species except for brook trout and Arctic char with *Ssa289* ($n = 3$) and for Atlantic salmon ($n = 146$). Allele size range is given in base pairs.

[a] Tetraploid locus.

reported in a number of studies (Slettan et al. 1993; Brooker et al. 1994). In humans and rats, the most common microsatellite repeat size is 12–15 and 14–25 repeat units, respectively, with 14% of human microsatellite sequences >20 dinucleotide units long and 6% of rat microsatellite sequences >30 dinucleotides long (Beckman and Weber 1992). Similar results have been reported for mice (Love et al. 1990), dogs (Ostrander et al. 1993), and pigs (Wintero et al. 1992). In Atlantic salmon we observed that 44% of microsatellite sequences were less than 13 dinucleotides in length and 18% were greater than 40, with 9 repeats (3.9%) consisting of 60 or more repetitive units. We and Slettan et al. (1993) found the frequency of microsatellites with greater than 24 repeat units in Atlantic salmon to be twice as high as in mice and seven times as high as in pigs and humans. In cod, Brooker et al. (1994) found that the second most common microsatellite repeat size class, 30–35 repeats, is twice the size of that reported for mammals and suggest that the longer microsatellite arrays found in teleost fishes may be due to an effect of temperature on DNA replication. In this study, 6% of microsatellite sequences isolated were found to be compound. Slettan et al. (1993) did not observe any compound sequences; however, they observed fewer microsatellite loci.

### Genetic variation among populations

The development of markers exhibiting a wide range of polymorphism and heterozygosity values is essential for application to studies in fisheries and aquaculture (Wright and Bentzen 1994). The three microsatellite loci *Ssa4*, *Ssa14*, and *Ssa289* differ in terms of their observed heterozygosity and in the total number of alleles observed. Primers to locus *Ssa4* amplified a total of 34 discrete alleles and gave heterozygosities (observed) of 0.65–0.89 in the five populations of Atlantic salmon surveyed. Primers to locus *Ssa14*, however, amplified only four alleles with observed heterozygosities of 0.35–0.56. Locus *Ssa289* was intermediate in character between the other two loci, with seven alleles observed and heterozygosities of 0.3–0.75. Estoup et al. (1993) report five to seven alleles per locus for three microsatellite loci in brown trout, while at cod microsatellite loci, 1–48 alleles per locus were observed in 127 fish (Brooker et al. 1994).

Genetic distances (Nei 1978), calculated for loci *Ssa4*, *Ssa14*, and *Ssa289* for all populations, correlated with geographic distance. Loci *Ssa4* and *Ssa289* can discriminate both between populations and between continents. *Ssa14* showed no discrimination at the level of continent of origin but was able to discriminate between Bay of Fundy and eastern shore populations in Nova Scotia. Inner Bay of Fundy Atlantic salmon populations are known to differ from other Maritime populations because they are comprised largely of grilse (Mr. G. Farmer, D.F.O., Halifax, personal communication) which stay within the Bay of Fundy rather than migrating to feeding grounds off Greenland. Previous work has also discriminated between Atlantic salmon of North American and European origin based on scale meristics (Reddin 1986), allozymes (Verspoor 1988), mitochondrial DNA restriction patterns (Bermingham et al. 1991), and a polymorphism in the ribosomal RNA gene (Davidson et al. 1989; Cutler et al. 1991). Microsatellite markers may enable rapid and reliable determination of continent of origin of salmon caught in the west Greenland mixed fishery. The ability to use minute, alcohol-preserved tissue samples and (or) fresh or archival scale samples circumvents problems of sample collection (Verspoor 1988). Use of scale samples would allow the calibration of scale analyses (Reddin 1986) by surveying the same sample set with an independent technique.

Studies in other organisms have demonstrated the potential of using microsatellites to distinguish among populations (Edwards et al. 1992; Estoup et al. 1993; Bowcock et al. 1994; Gotteli et al. 1994; Morin et al. 1994). The present study demonstrates that a similar potential exists for discrimination among populations of Atlantic salmon using microsatellite markers. The level of discrimination possible has yet to be fully explored.

### Amplification of homologous microsatellite loci in other salmonine species

A particularly exciting finding from this study is the ability of the primers for loci *Ssa4*, *Ssa14*, and *Ssa289* to amplify presumably homologous microsatellite loci in a wide range of salmonine species. This is of importance considering the high initial cost of developing markers for a particular species. The level of polymorphism at microsatellite loci in salmonids seems to be largely conserved across species. We have previously observed that primers developed for two microsatellite loci isolated from rainbow trout (Marine Gene Probe Laboratory, unpublished data) permit amplification of polymorphic microsatellites in Atlantic salmon (S.K. McConnell, unpublished data). Estoup et al. (1993) also noted that primers developed to amplify microsatellite loci in brown trout could also amplify microsatellite loci in rainbow trout. Primers developed for Atlantic cod also permitted amplification of loci in a wide range of other gadiid species (Brooker et al. 1994). In mammals, primers developed for cattle have been shown to amplify microsatellite loci in sheep and horses (Moore et al. 1991).

Microsatellite loci make ideal genetic markers for population studies because they are abundant, show varying degrees of allelic variation, and can be rapidly assayed by PCR from minute amounts of tissue. Other applications for microsatellite markers in salmon include genome mapping, the assessment of inbreeding in aquaculture populations (Gjedrem 1992) and in pedigree analysis, and the assessment of reproductive success both in aquacultural and natural situations.

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# Comparative growth and survival patterns of *Mytilus trossulus* and *Mytilus edulis* in Atlantic Canada

**André L. Mallet and Claire E. Carver**

**Abstract**: Two size groups (20–30 and 45–55 mm) of *Mytilus edulis* and *Mytilus trossulus* were assessed from May to December 1992 at two locations in Nova Scotia. The two species were classified using electrophoresis based on mannose phosphate isomerase, and a discriminant function based on shell morphometry. The discriminant function misclassified less than 5% of the mussels and of these, 1% of the *M. edulis* were misclassified as *M. trossulus*, and 10% of the *M. trossulus* were misclassified as *M. edulis*. In general, both size groups of *M. edulis* tended to have higher shell growth rates than *M. trossulus*. Overall mortality was 6% and occurred primarily from mid-August to mid-September; *M. edulis* exhibited a slightly lower survival and for a given shell length had a higher average tissue and shell weight, as well as a greater shell height, than *M. trossulus*. *Mytilus trossulus* had a significantly lower tissue weight during the summer and was eliminated at a significantly higher rate during commercial grading trials than *M. edulis*. We estimated from the information on individual weight, survival, and grading losses that the economic value of *M. edulis* was 1.7 times higher than that of *M. trossulus*.

**Résumé** : Nous avons évalué deux groupes de taille (20–30 et 45–55 mm) de *Mytilus edulis* et de *Mytilus trossulus*, de mai à décembre 1992, à deux endroits de la Nouvelle-Écosse. Nous avons séparé les deux espèces par électrophorèse à partir de la phospho-mannose-isomérase et par une fonction discriminante fondée sur la morphométrie de la coquille : la fonction discriminante a mal classé moins de 5% des moules et, parmi celles-ci, 1% des *M. edulis* ont été classées à tort comme les *M. trossulus*, tandis que 10% des *M. trossulus* ont été classées comme les *M. edulis*. De façon générale, dans les deux groupes de taille, *M. edulis* présentait un taux de croissance de la coquille plus élevé que *M. trossulus*. La mortalité était globalement de 6%; elle se produisait surtout de la mi-août à la mi-septembre; *Mytilus edulis* présentait un taux de survie légèrement plus bas et, pour une longueur de coquille donnée, avait un poids moyen de tissus et de coquille plus élevé, ainsi qu'une hauteur de coquille plus grande que *M. trossulus*. *Mytilus trossulus* avait pendant l'été un poids de tissus significativement plus bas et a été éliminée pendant les essais de tri commercial à un rythme significativement plus élevé que *M. edulis*. À partir de l'information sur le poids individuel, la survie et les pertes au tri, nous estimons que la valeur économique de *M. edulis* est 1,7 fois plus élevée que celle de *M. trossulus*.

[Traduit par la Rédaction]

## Introduction

Several studies have established the presence of two species of blue mussels, *Mytilus edulis* and *Mytilus trossulus*, in certain regions of Atlantic Canada (Varvio et al. 1988; McDonald et al. 1991; Gosling 1993). Specifically, mixed populations of the two species with varying proportions are a common occurrence along the Atlantic coast of Nova Scotia, in Newfoundland, and along the upper reaches of the Gulf of St. Lawrence. Pure populations of *M. edulis* are found along the Northumberland Strait and in Prince Edward Island, whereas pure populations of *M. trossulus* are found in the Bras d'Or Lakes (Cape Breton), Nova Scotia (A.L. Mallet, unpublished data). These mussel species interbreed successfully under hatchery conditions (Zouros et al. 1992) and biparental mtDNA inheritance is common in wild populations (Hoeh et al. 1991).

Little is known about the relative performance of *M. edulis* and *M. trossulus* under natural conditions. Freeman et al. (1992) suggested that, although *M. edulis* tended to produce a greater quantity of gametes, the reproductive cycles of the two species, as inferred from condition and gametogenic indices, showed nearly synchronous spawning. At present, several commercial mussel farms are operating successfully with a mixed seed stock of *M. edulis* and *M. trossulus*. Certain operators argue, however, that higher production rates could be achieved if they were to grow a pure stock of *M. edulis*. This study focuses

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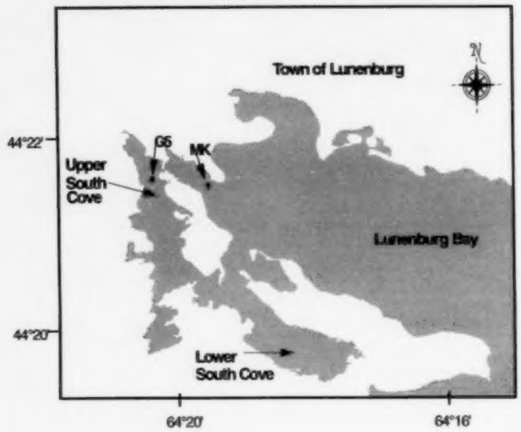
**Fig. 1.** Map of Lunenburg Bay indicating the position of the two sampling locations, G5 in Upper South Cove and MK in Lunenburg Bay.



on (*i*) comparing the growth and survival patterns of *M. edulis* and *M. trossulus* each grown in two environments and (*ii*) evaluating the effects of commercial processing on the yield of these two species of blue mussels.

## Materials and methods

### Location of study

The study was carried out at a commercial mussel operation located near Lunenburg, Nova Scotia, Canada, between May 14 and December 8, 1992. This site has previously been described in other studies (Mallet and Carver 1991; Grant et al. 1993; Mallet and Carver 1993) and was known to have approximately equal proportions of *M. trossulus* and *M. edulis* in the resident mussel population (C.E. Carver and A.L. Mallet, unpublished data). Mussels were deployed at two locations (Fig. 1) to expose them to different temperature regimes; for example, an extensive environmental monitoring program (1989 to 1991) had indicated that the "G5" location was consistently warmer than the "MK" one (Mallet and Carver 1993).

### Experimental design

Mussels used to estimate individual shell growth and mortality were obtained from commercial mussel sleeves grown at each location. Two size groups were selected, 20–30 and 45–55 mm, because they had been shown to exhibit differential growth and mortality profiles (Mallet and Carver 1993). The experimental unit consisted of a strip of mosquito mesh, 3 m long by 15 mm wide, onto which 10 pairs of mussels were fixed back-to-back using 5-min Epoxy glue. Every 4 weeks between May 14 and October 27, 1992, eight experimental units were prepared as follows: two size classes (20–30 and 45–55 mm), two stations (MK and G5), and two replicated strips per station for a total of 160 mussels (2 stations × 2 size groups × 2 replicates × 20 mussels). Each individual was measured (shell length, height, and width) and the number of survivors was counted every 2nd week for 6 weeks. At the end of this period, the mussels were dissected and their hepatopancreas tissues were frozen at $-40$°C for subsequent electrophoretic analysis. Cellulose acetate electrophoresis was used to separate allozymes of mannose-6-phosphate isomerase (MPI, EC 5.3.1.8) in Tris–glycine buffer (Hebert and Beaton 1989). The MPI allozyme can be considered diagnostic for distinguishing between *M. edulis* and *M. trossulus* (Gosling 1993, pp. 318–319). Shells from the dead mussels were measured and a discriminant analysis was used to determine species identity.

### Dry weight measurements

The mussels used for the dry tissue and shell weight profiles during this study were sampled from mussel sleeves deployed at each of the experimental locations on May 14, 1992. Fifty mussels ranging in size from 15 to 60 mm were collected at each location every 2 weeks for a total of 15 collections. These were dissected, dried at 60°C for 48 h, measured, and weighed to determine shell length, dry shell weight, and dry tissue weight. Each shell was labelled and individuals were later classified as *M. edulis* or *M. trossulus* on the basis of their shell morphology.

### Morphometric measurements

Following the morphometric approach of McDonald et al. (1991), those shell characters that had a high absolute standardized canonical coefficient were used to classify individuals as *M. edulis* or *M. trossulus* (Fig. 2). The characters were as follows: (*i*) the length of the anterior adductor muscle scar (AAM); (*ii*) the length of the hinge plate (HP); (*iii*) the distance between the anterior edge of the posterior adductor muscle scar and the shell margin (PADP); (*iv*) the distance between the ventral edge of the posterior adductor muscle scar and ventral shell margin (PADV); (*v*) the distance between the pallial line and the ventral shell margin midway along the shell (PAL); (*vi*) shell height (HEI); and (*vii*) shell width (WID). Each character was standardized using $\log_{10}$ and divided by the $\log_{10}$ of shell length. A canonical discriminant analysis was used to derive a canonical function that separated the two mussel types (Candisc, SAS, V6.08). The parameters were also used to generate a canonical variate for each of the individuals which could not be assessed electrophoretically. The *F* statistic indicated whether a character by itself contributed significantly to the distinction between the two species, whereas the absolute value of the standardized canonical coefficient indicated the relative contribution of each character to the separation of the two species.

### Effects of commercial processing

The effect of processing on the ratios of the two species was evaluated at a mussel processing plant located near the town of Mahone Bay, Nova Scotia. Two samplings were carried out: June 16, 1992, and March 31, 1993. Four samples of 50 mussels each were taken on each sampling date: (*i*) unprocessed mussels; (*ii*) undersized mussels which had fallen through the rings of the grader; (*iii*) market-size mussels rejected because of damaged shells; and (*iv*) mussels ready for market. In the first sampling, the two mussel types were identified on the basis of their electrophoretic genotypes, and in the second sampling, on the basis of their shell characteristics.

**Table 1.** Probability from *F* statistic and the standardized canonical coefficients for the seven morphological characters used in the discriminant analysis.

| Character | Probability | Standardized coefficient |
|---|---|---|
| AAM | <0.01 | 1.03 |
| HP | <0.01 | 0.60 |
| PAL | <0.01 | −0.12 |
| PADP | 0.89 | −0.21 |
| PADV | 0.05 | −0.46 |
| HEI | 0.00 | 0.66 |
| WID | 0.02 | −0.28 |

**Note**: AAM, length of the anterior adductor muscle scar; HP, the length of the hinge plate; PADP, distance between anterior edge of posterior adductor muscle scar and shell margin; PADV, distance between ventral edge of posterior adductor muscle scar and ventral shell margin; PAL, distance between pallial line and ventral shell margin midway along the shell; HEI, shell height; WID, shell width.

### Data analysis

The shell growth data file consisted of the shell length, width, and height of mussels sampled at different times and genotyped electrophoretically. Individual growth values were estimated by regressing the measured character versus time and using the slope of the regression as the growth rate. No partial growth records were used; mortalities resulted in the elimination of these records from the growth dataset. To test for locations and species effects, the growth values were analyzed with a mixed effect ANOVA model as follows:

$$Y_{ijk} = \mu + T_i + L_j + S_k + LS_{jk} + n_{ijk} + \rho x_{ijkl} + e_{ijkl}$$

where $\mu$ is the population mean; $T_i$ is a random effect as a result of the *i*th time period, $i = 1, \ldots, 7$; $L_j$ is a fixed effect as a result of the *j*th location, $j = 1, 2$; $S_k$ is a fixed effect as a result of the *k*th species, $k = 1, 2$; $n_{ijk}$ is a random effect consisting of 2nd and 3rd order interactions with time that was used as the experimental error; $\rho x$ is a covariate as a result of the initial measurement of a given time period; and $e_{ijkl}$ is a residual peculiar to the *l*th growth values in the *i*th time interval at the *j*th location for the *k*th species. Least square means were also obtained from these analyses.

Dead mussels were classified as *M. edulis* or *M. trossulus* on the basis of their shell morphology. The data were read as number of events and number of withdrawals at each time interval. Species and location effects were tested separately by the log rank test as used in Mallet et al. (1990).

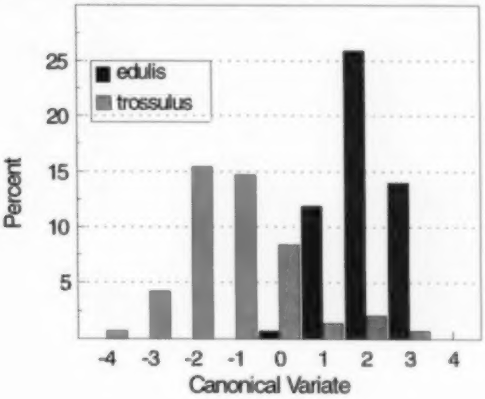
Separate regression equations for shell weight, shell height, shell width, and tissue weight versus shell length were obtained for each of the two species at each location and sampling event. Values for a standardized 30- and 55-mm mussel were predicted from quadratic regressions (see also Mallet and Carver 1993) and used in an ANOVA to evaluate differences between locations and species.

## Results

### Discriminant analysis on morphometric characters

Seven shell characters were used to effectively discriminate (Wilk's lambda: $p < 0.0001$) between individuals that had previously been identified electrophoretically as *M. edulis* or *M. trossulus* (Table 1). Positive canonical coefficients were obtained when the measurements for *M. edulis* were greater than those for *M. trossulus*. As noted by McDonald et al. (1991), the lengths of the adductor muscle scar and the hinge plate were very important in the discriminant function. The analysis also indicated that shell height was an important measurement (see also Freeman et al. 1992). PADP did not have any discriminatory power on its own ($p = 0.89$), although as part of the discriminant function, it contributed more than PAL (as depicted by the absolute value of the canonical coefficient). The positive value of the standardized canonical coefficients indicated that *M. edulis* had a longer anterior adductor mussel scar, a longer hinge plate, and a greater shell height than *M. trossulus*.

**Fig. 2.** Canonical variate distribution for *M. edulis* and *M. trossulus* individuals of known genotypes.



### Accuracy of electrophoretic scoring and morphometric measurements

The ability of the discriminant function to distinguish between the two species was assessed by comparing the individual canonical variates of 143 mussels with their electrophoretic scores (Fig. 2). The distribution of the canonical variates (Fig. 2) showed some degree of overlap between the two species, but in general, the separation was quite clear. A small fraction of this error may be related to the electrophoretic procedures; there is always the possibility that errors may have occurred in the labelling or dissection of the samples, or during the loading of the plates. On the basis of morphology, 7 of the 143 mussels were misclassified for a total of 4.9%. Of the seven mussels that were misclassified, four were market size and three were less than 30 mm; hence, size did not appear to be a factor in the misclassification. On the other hand, six of the seven misclassified mussels were *M. trossulus*, which had been classified as *M. edulis* on the basis of shell characters. This would suggest that approximately 10% of *M. trossulus* mussels appear to look like *M. edulis*, whereas approximately 1% of *M. edulis* mussels resemble *M. trossulus*.

The accuracy of the discriminant analysis based on 7 characters was comparable to that of McDonald et al. (1991) who used 17 characters. Trials with various subsets

**Table 2.** Mean squares (MS) and probabilities ($p$) from the mixed model ANOVA for the growth of 20–30 and 45–55 mm mussels in terms of shell length, height, and width evaluating the effect of time, location, and species.

| | | Length | | Height | | Width | |
|---|---|---|---|---|---|---|---|
| Source | df | MS | $p$ | MS | $p$ | MS | $p$ |
| | | **20–30 mm mussel** | | | | | |
| Time ($T$) | 6 | 0.0162 | 0.10 | 0.0043 | 0.19 | 0.0017 | 0.14 |
| Location ($L$) | 1 | 0.0000 | 0.99 | 0.0002 | 0.77 | 0.0010 | 0.27 |
| Species ($S$) | 1 | 0.0000 | 0.99 | 0.0037 | 0.24 | 0.0005 | 0.43 |
| $L \times S$ | 1 | 0.0028 | 0.54 | 0.0021 | 0.37 | 0.0000 | 0.97 |
| SE | 18 | 0.0091 | <0.01 | 0.0031 | <0.01 | 0.0010 | <0.01 |
| Covariate | 1 | 0.0324 | <0.01 | 0.0001 | 0.93 | 0.0024 | 0.01 |
| Error | 414 | 0.0021 | | 0.0009 | | 0.0003 | |
| | | **45–55 mm mussel** | | | | | |
| Time ($T$) | 6 | 0.0045 | 0.11 | 0.0015 | 0.75 | 0.0005 | 0.07 |
| Location ($L$) | 1 | 0.0009 | 0.53 | 0.0021 | 0.39 | 0.0001 | 0.38 |
| Species ($S$) | 1 | 0.0068 | 0.10 | 0.0016 | 0.39 | 0.0011 | 0.02 |
| $L \times S$ | 1 | 0.0023 | 0.31 | 0.0000 | 0.99 | 0.0006 | 0.09 |
| SE | 18 | 0.0024 | <0.01 | 0.0029 | <0.01 | 0.0002 | 0.25 |
| Covariate | 1 | 0.0061 | 0.02 | 0.0013 | 0.32 | 0.0006 | 0.04 |
| Error | 412 | 0.0012 | | 0.0013 | | 0.0002 | |

**Note**: SE is the experimental error that is constructed from 2nd and 3rd order interactions of TIME with the other main effects. SE is used in the $F$ statistics as the denominator for $T$, $L$, $S$, and $L \times S$. The covariate is the initial shell measurement for the character. Growth was estimated from the slope of the regression for each mussel over the sampling interval.

of characters showed that the combination of AAM, HP, WID, and HEI provided discriminative results similar to the full set of characters, whereas the combination of HP, AAM, PADP, PADV, and PAL nearly doubled the probability of misclassifying individuals. Mussels sampled from natural sites, such as exposed beaches or rock crevices, may show a higher degree of morphological variability than cultured mussels, which tend to experience less variable conditions. At present, the major drawback of this morphological assessment is the inability to identify hybrid mussels. A large sample of electrophoretically identified hybrids would be required to determine whether these mussels are morphologically distinct from pure *M. edulis* or *M. trossulus*.

### Shell growth

The effect of time, location, and species on growth in terms of shell length, height, and width was evaluated by means of an analysis of variance on individual growth values (Table 2). The mixed model ANOVA effectively acts as a paired test; rather than evaluating for an overall growth difference between species, it tests whether the mean difference between species among time periods is consistent in direction and magnitude. For the 20–30 mm size group, no significant differences were detected between species (Table 2); however, if it could be assumed that the $\sigma^2_{\mathrm{TLS}} = 0$, then growth in the height dimension was significantly different between species. For the 45–55 mm group, growth in the width dimension was statistically different between species (Table 2); however, if it could be assumed that the $\sigma^2_{\mathrm{TLS}} = 0$, then growth in both the length and the width dimensions was significantly different between species. For example, in the case of the larger mussels, *M. edulis* grew on average 12 μm/day (±0.007) faster than *M. trossulus*. In general, the least square mean values for growth of most characters in both size groups were higher for *M. edulis* than *M. trossulus* (Fig. 3).

No significant temporal variations (TIME) were detected in the length, height, and width growth values for the 20–30 and 45–55 mm mussels (Table 2). In addition, there was no spatial effect (LOCATION); however, growth values for the larger mussels tended to be higher at MK than at G5 (Fig. 3).

### Survival

The overall survival level in this study, estimated across locations and species, was 94%. Survival levels were not statistically different between locations for both size groups. Differences in survival were only significant (Wilcoxon: $p = 0.02$) between large *M. edulis* (90% overall) and *M. trossulus* (95%) (Fig. 4). Most of the mortality was observed from mid-August to late September (data not shown).

### Shell and tissue measurements

Quadratic relationships (e.g., tissue weight vs. shell length) derived for each species at each location and sampling date (every 2 weeks) had coefficients of determination ($r^2$) typically greater than 0.85 (df = 49), and most of the linear and quadratic terms were significant. The quadratic

**Table 3.** Mean squares (MS) and probability ($p$) from an ANOVA evaluating the effect of location, species, and their interactions on tissue weight, shell weight, shell height, and shell width for a 30- and a 55-mm mussel.

| | | Tissue wt. | | Shell wt. | | Height | | Width | |
|---|---|---|---|---|---|---|---|---|---|
| Source | df | MS | $p$ | MS | $p$ | MS | $p$ | MS | $p$ |
| **30-mm mussel** | | | | | | | | | |
| Location ($L$) | 1 | 0.020 | 0.01 | 0.0040 | 0.690 | 0.38 | 0.48 | 4.40 | 0.02 |
| Species ($S$) | 1 | 0.0070 | 0.13 | 0.5980 | <0.01 | 14.80 | <0.01 | 1.50 | 0.15 |
| $L \times S$ | 1 | 0.0060 | 0.16 | 0.0020 | 0.78 | 0.01 | 0.91 | 1.00 | 0.24 |
| Error | 58 | 0.0030 | | 0.0250 | | 0.76 | | 0.70 | |
| **55-mm mussel** | | | | | | | | | |
| Location ($L$) | 1 | 0.027 | 0.23 | 4.76 | <0.01 | 0.86 | 0.40 | 2.70 | 0.07 |
| Species ($S$) | 1 | 0.345 | <0.01 | 14.4 | <0.01 | 98.30 | <0.01 | 1.00 | 0.27 |
| $L \times S$ | 1 | 0.019 | 0.31 | 0.003 | 0.91 | 0.40 | 0.56 | 0.20 | 0.62 |
| Error | 58 | 0.018 | | 0.254 | | 1.18 | | 0.79 | |

**Fig. 3.** Least square mean growth in length, height, and width for the 20–30 mm and 45–55 mm mussels averaged over the whole study.

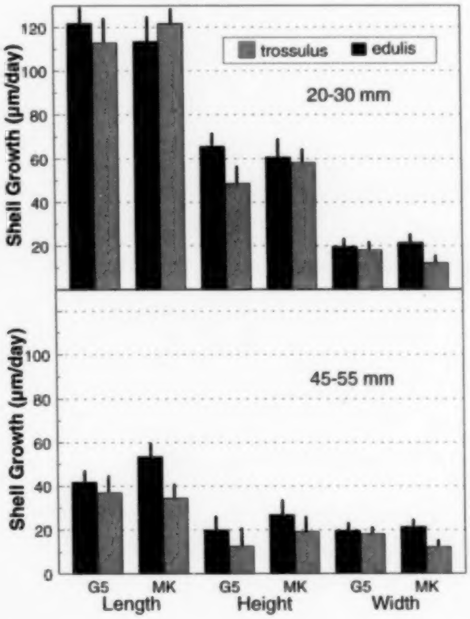
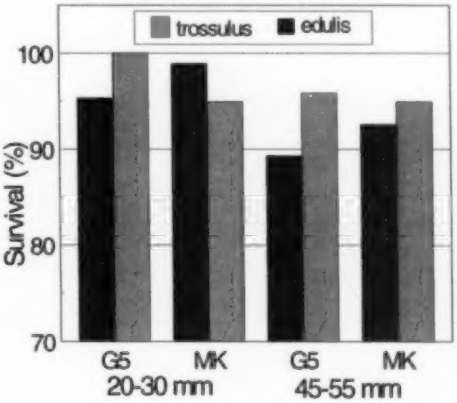


**Fig. 4.** Overall survival of *M. edulis* and *M. trossulus* in the 20–30 mm and the 45–55 mm size groups at G5 and MK.



equations were used to predict the tissue weight, shell weight, shell height, and shell width at different times for a standard 30- and 55-mm mussel. These values were then used in an ANOVA to test for overall location and species differences (Table 3).

One effect that was consistent over size groups was the greater shell weight and shell height of *M. edulis* compared with *M. trossulus* (Table 4). There was no significant variation in shell width between species or locations. These trends confirm the results of the morphological assessment that identified shell height as an important discriminating variable. In terms of shell weight, 55-mm *M. edulis* and *M. trossulus* can differ by as much as 1.5 g, depending on the location (Fig. 5). In terms of dry tissue weight, the two species differed on average by 0.18 g at G5 and 0.12 g at MK. In the summer, this difference was as much as 0.4 g (Fig. 6). Although these weight variations appear small, when dry weight is translated to wet weight, for example at G5, 143 *M. trossulus* mussels would be needed to make up 1 kg as opposed to only 111 *M. edulis*.

Although not shown in Fig. 6, there was no overlap in the confidence intervals for tissue weight during the summer. For example, 55-mm *M. edulis* tended to have a higher tissue weight than *M. trossulus* at both locations during the summer (Fig. 6), whereas spring and fall tissue weight values were similar for the two species (overlapping confidence intervals). The 30-mm *M. edulis* had a noticeably higher weight at G5 than at MK (Fig. 6). In general, tissue weight values were highest in the spring (after the bloom

**Table 4.** Least square means (with 1 SE) for tissue weight, shell weight, shell height, and shell width for a standard size 30- and 55-mm *M. trossulus* or *M. edulis* mussel at G5 or MK over all sampling times.

| Location | Species | Size (mm) | Tissue wt. (g) | Shell wt. (g) | Shell height (mm) | Shell width (mm) |
|---|---|---|---|---|---|---|
| G5 | ED | 30 | 0.20 (0.01) | 0.79 (0.04) | 16.5 (0.22) | 10.6 (0.21) |
| | | 55 | 0.92 (0.03) | 4.05 (0.13) | 28.1 (0.28) | 19.1 (0.23) |
| | TR | 30 | 0.16 (0.01) | 0.58 (0.04) | 15.5 (0.22) | 10.7 (0.21) |
| | | 55 | 0.74 (0.03) | 3.07 (0.13) | 25.7 (0.28) | 19.2 (0.23) |
| MK | ED | 30 | 0.14 (0.01) | 0.79 (0.04) | 16.6 (0.22) | 9.9 (0.20) |
| | | 55 | 0.85 (0.03) | 4.59 (0.13) | 28.5 (0.27) | 18.5 (0.22) |
| | TR | 30 | 0.14 (0.01) | 0.61 (0.04) | 15.7 (0.22) | 10.4 (0.20) |
| | | 55 | 0.73 (0.03) | 3.64 (0.13) | 25.8 (0.27) | 18.9 (0.22) |

**Fig. 5.** Dry shell weight of a standard 30-mm and 55-mm *M. edulis* or *M. trossulus* mussel at G5 and MK over time.

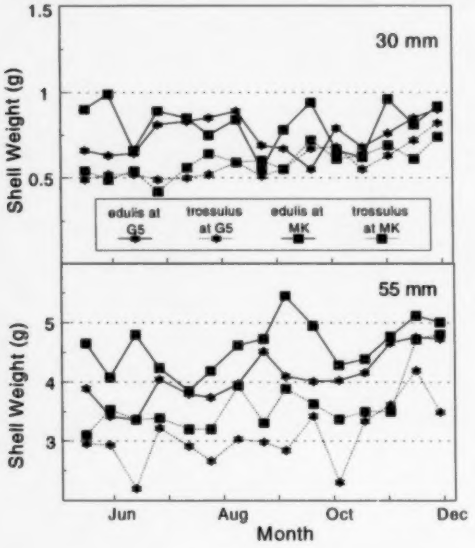
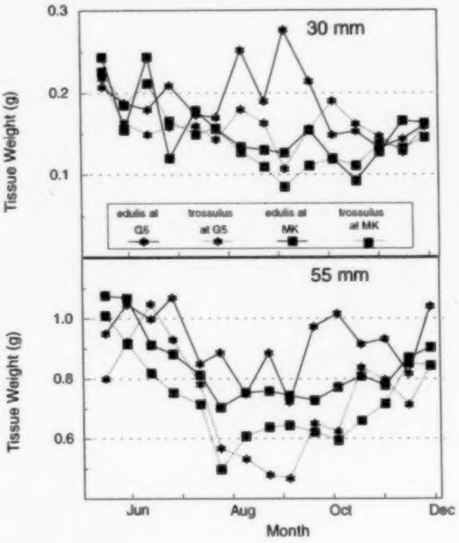


**Fig. 6.** Dry tissue weight of a standard 30-mm and 55-mm *M. edulis* or *M. trossulus* mussel at G5 and MK over time.



but before spawning) and the fall (following the phytoplankton bloom). Profiles for 30-mm mussels indicated that tissue weight declined from May to June and then stabilized, whereas the 55-mm mussels showed signs of rebuilding in October. No significant temporal trends were observed for shell weight; however, non-overlapping confidence intervals consistently suggested higher values for *M. edulis* than *M. trossulus*.

### Effects of processing

Mussels harvested from Indian Point Mussel Farms near Mahone Bay, Nova Scotia, were sampled prior to grading in June 1992 and March 1993 (Table 5). The ratio of *M. edulis* to *M. trossulus* in the unprocessed sample differed between sampling events. On both occasions, however, there was a substantial increase in the proportion of *M. trossulus* in the undersized, or submarket, group. A similar increase in the proportion of *M. trossulus* relative to *M. edulis* was evident in the broken shell group in March 1993, although not in June 1992. There was a slight increase in the ratio of *M. edulis* to *M. trossulus* in the market-size group in June, but no change was noted in March.

## Discussion

In general, shell growth values were higher for *M. edulis* than for *M. trossulus* (Fig. 3), although only one statistically significant difference is detected by the mixed model ANOVA. Both the discriminant analysis of shell measurements (Table 1) and the shell height estimates (Tables 3 and 4) clearly showed that for a given shell length, *M. trossulus* had a significantly lower shell height than *M. edulis*. In a commercial context, the narrower shape (i.e., lower shell height) of *M. trossulus* seemed to increase the probability of their falling through the rings of the grader.

The analysis of shell and tissue weight indicated a significant overall variation between locations and species. For example, a 1.5 g mean difference was noted between the shell weight of 55-mm *M. trossulus* grown at G5 (3.07 g) and 55-mm *M. edulis* grown at MK (4.59 g). Note that the temporal profile of shell weight (Fig. 5) showed unexplained variability between sampling events, although these were not statistically significant. In terms of dry tissue weight, 55-mm *M. edulis* grown at G5 weighed 0.18 g more (approximately 0.90 g wet weight) than 55-mm *M. trossulus*. Temporal profiles of tissue weight for a 55-mm mussel showed that (*i*) the decline in weight as a result of spawning occurred simultaneously in the two species; (*ii*) the two species had similar tissue weights prior to spawning; and (*iii*) *M. trossulus* had half the tissue weight of *M. edulis* during the summer. Freeman et al. (1992) documented similar tissue differences in terms of condition and gametogenic indices between species. In some instances, this difference in meat yield between species during the summer may cause producers to delay harvesting until the fall (P. Darnell, mussel grower, personal communication).

Analyses comparing the survival of the two species over the two locations showed that *M. trossulus* had a slightly higher survival (95%) than *M. edulis* (90%). This difference was primarily due to the higher mortality of *M. edulis* at the warmer G5 location. Instances of high mortality in *M. edulis* during the summer are well documented (Mallet and Carver 1991) and may be as high as 40%; by comparison, the overall values observed in this study were relatively low. Because of the predominance of *M. edulis* in the Northumberland Strait area (MacDonald et al. 1991), it was expected that this species would have a higher survival than *M. trossulus* at the warmer, more estuarine G5 location. However, it seems that *M. trossulus* can tolerate higher temperatures as well as lower salinities (Gosling 1993) better than *M. edulis*. Given that the two species also appear to have similar reproductive patterns, another explanation is required to account for the exclusion of *M. trossulus* from Prince Edward Island and the east coast of New Brunswick.

Previous work has shown that adjacent populations of pure *M. edulis* can exhibit significantly different growth and survival profiles (Mallet and Carver 1991). Mixed populations of *M. edulis* and *M. trossulus* commonly occur along the Atlantic coast of Nova Scotia, Canada (A.L. Mallet, personal observation). To our knowledge, however, the reported values are the first published data comparing the growth and survival of these two mytilid species. Personal observations and anecdotal evidence from various mussel producers suggest that the relative performance of the two species in this study was consistent with their performance at other sites with mixed populations. However, because no replicate populations were examined, the consistency of this ranking was not determined.

Another area of interest is the apparent scarcity of hybrids at a site where the two mussel species show concurrent reproductive activity. The apparent synchrony in the spawning cycle of the two species (see also Freeman et al. 1992) is similar to that described for mixed populations of *Mytilus galloprovincialis* and *M. edulis* in Ireland (Gosling and Wilkins 1981). Apparently, whenever these two mussel species are found together, they do hybridize, sometimes extensively. In the present study, approximately 10 hybrids were identified electrophoretically (<2%), whereas the expectation should be around 40%. Hybrid *M. edulis* and *M. trossulus* can be produced artificially (Zouros et al. 1992), although it is not yet known whether these hybrids are fertile and whether they show survival patterns similar to the within-species crosses.

**Table 5.** Ratios of *M. edulis* and *M. trossulus* at different stages during the grading process at two sampling events.

| | June 1992 (*edulis*:*trossulus*) | March 1993 (*edulis*:*trossulus*) |
|---|---|---|
| Initial | 18:82 | 48:52 |
| Undersized | 3:97 | 28:72 |
| Broken | 15:85 | 17:83 |
| Market | 28:72 | 45:55 |

**Note**: Each ratio was computed from a total of 50 mussels.

From a commercial perspective, the coexistence of these two mytilid species would be of little concern given that the two forms had similar production performance. The evidence presented in this study, however, suggests that *M. edulis* would provide a higher return on investment than *M. trossulus*. To further test this hypothesis, the individual weight and survival data were used to calculate the relative yield when growing similar numbers of each species. The following conditions were assumed: (*i*) 20% of the initial harvest weight is typically rejected during the grading process (P. Darnell, personal communication); (*ii*) given an initial ratio of 50% *M. trossulus* to 50% *M. edulis*, 20% of the *M. edulis* and 80% of the *M. trossulus* are rejected during grading (Table 5); (*iii*) survival to market size is 95% for *M. trossulus* and 90% for *M. edulis*, (*iv*) the whole wet weight of *M. edulis* is 9 g compared with 7 g for *M. trossulus*, and (*v*) 1800 mussels are initially stocked in a 3-m sleeve.

Under these conditions, 1 kg of unprocessed mussels would contain 125 mussels (62.5 *M. edulis* at 9 g and 62.5 *M. trossulus* at 7 g) of which 0.2 kg (20% of initial harvest) or 27 mussels would be rejected (5.4 *M. edulis* (20% of 27) compared with 21.6 *M. trossulus* (80% of 27)). The grading procedure thus eliminates 8.6% of *M. edulis* (5.4/62.5) and 34.3% of *M. trossulus* (21.6/62.5); alternatively, we can state that 91.4% of the *M. edulis* and 65.7% of the *M. trossulus* are retained during the grading process. The yield of one sleeve of pure *M. trossulus* can then be computed as follows: an initial stocking density of 1800 mussels, multiplied by a survival rate of 0.95, multiplied by a retainment rate of 0.657, multiplied by an individual weight of 7 g would give a final yield of 7.9 kg. Similarly, for the same initial stocking density, a total weight of 13.3 kg of *M. edulis* would be achieved (1800 × 0.90 × 0.914 × 9 g). Thus, the combination of losses during grading and differences in individual weight would mean that the initial number of *M. trossulus* must be increased by a factor of 1.7 to achieve the same return as

*M. edulis*. Obviously, growing mixtures of the two species would tend to reduce the disparity in the final yield (see also Mallet and Carver 1991).

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# Temporal and environmental variation in the biomass spectrum of benthic invertebrates in streams: an application of thin-plate splines and relative warp analysis

## Antoine Morin, Marco A. Rodríguez, and Daniel Nadon

**Abstract**: The size distribution of benthic invertebrates found on the surface of rocks was examined in 12 streams of the Ottawa Valley for 15 months. We used relative warp analysis and thin-plate splines to demonstrate that normalized biomass spectra were similar among sampling dates and among sites, although statistically significant seasonal patterns were detected. The relative abundance of the smallest organisms (mainly early instars of chironomid larvae between 0.1 and 1 mm long) varied the most and peaked in early summer in warm waters, leading to a reduction of the average size of invertebrates in early summer. Overall abundance also positively but weakly correlated with periphyton standing stock. Average size was larger in urban streams of Ottawa–Hull than in streams in cottage areas of the Outaouais or draining the protected forests of Gatineau Park, even after accounting for the effect of periphyton standing stock and concentration of total phosphorus. Total community production, estimated using the average size distribution in all sites and a published empirical model predicting instantaneous growth rate, peaked in early summer and reached a minimum in winter, following closely the seasonal water temperature pattern. The contribution of organisms smaller than 1 mm to total community production was only significant in early summer when it reached 20%.

**Résumé** : La distribution selon la taille des invertébrés benthiques retrouvés à la surface des roches a été examinée dans 12 ruisseaux de la vallée de l'Outaouais pendant 15 mois. Nous avons utilisé l'analyse du gauchissement relatif (« relative warp ») et les splines à couche mince (« thin-plate splines ») pour démontrer que, quoique les spectres normalisés de biomasse soient similaires dans tous les sites et à toutes les dates d'échantillonnage, il existe une tendance saisonnière. L'abondance relative des organismes les plus petits (principalement des jeunes stades larvaires de chironomides de 0,1 à 1 mm de longueur) est particulièrement variable et atteint un sommet au début de l'été en eaux chaudes, entraînant une réduction de la taille moyenne des invertébrés au début de l'été. L'abondance totale est positivement mais faiblement corrélée à la biomasse du périphyton. La taille individuelle moyenne est plus élevée dans les ruisseaux de la zone urbaine d'Ottawa–Hull que dans les ruisseaux de la zone de villégiature de l'Outaouais ou dans ceux qui drainent les forêts du Parc de la Gatineau, et ce même après correction de l'effet de la biomasse du périphyton et de la concentration totale en phosphore sur la taille individuelle. La production totale de la communauté, estimée à partir de la distribution moyenne selon la taille et d'un modèle empirique prédisant le taux instantané de croissance, est maximale en été et minimale en hiver, et suit de près les variations saisonnières de température. La contribution des organismes de moins de 1 mm à la production totale de la communauté n'est significative qu'au début de l'été lorsqu'elle atteint 20%.

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**Table 1.** Sample locations, annual average width and depth of sampled sections, midsummer canopy cover, and average total phosphorus, seston, and sestonic chlorophyll concentrations in the water.

| Site | Location | Width (m) | Depth (m) | Canopy cover (%) | [TP] ($\mu g \cdot L^{-1}$) | [Seston] (mg $DM \cdot L^{-1}$) | [Chl *a*] ($\mu g \cdot L^{-1}$) |
|---|---|---|---|---|---|---|---|
| Ottawa–Hull | | | | | | | |
| Des Trembles | 45°26′N, 75°46′W | 3.5 | 0.2 | 0 | 90.7±17.3 | 28.5±6.0 | 4.4±0.8 |
| Green | 45°25′N, 75°35′W | 7.5 | 0.3 | 0 | 67.8±10.5 | 18.0±3.5 | 6.2±1.0 |
| Leamy | 45°27′N, 75°45′W | 3.5 | 0.3 | 0 | 128.5±48.7 | 125.6±62.2 | 3.3±0.7 |
| Sawmill | 45°23′N, 75°40′W | 5.0 | 0.2 | 100 | 61.2±6.1 | 22.0±2.6 | 4.7±0.9 |
| Outaouais | | | | | | | |
| Blackburn | 45°39′N, 75°49′W | 5.0 | 0.3 | 30 | 10.2±1.3 | 1.0±0.1 | 1.2±0.2 |
| Corriveau | 45°41′N, 75°44′W | 3.5 | 0.2 | 30 | 12.7±2.2 | 1.1±0.3 | 0.6±0.1 |
| Pélissier | 45°39′N, 75°44′W | 4.0 | 0.4 | 50 | 15.1±1.7 | 1.9±0.3 | 1.6±0.4 |
| Rainville | 45°35′N, 75°39′W | 9.5 | 0.2 | 0 | 15.3±2.6 | 1.3±0.2 | 7.9±5.7 |
| Gatineau Park | | | | | | | |
| Chelsea | 45°30′N, 75°48′W | 8.5 | 0.3 | 70 | 18.2±2.1 | 2.3±0.2 | 2.3±0.4 |
| Lapêche Sud | 45°39′N, 76°01′W | 4.0 | 0.2 | 0 | 17.3±2.9 | 2.0±0.3 | 1.8±0.2 |
| Renaud | 45°36′N, 76°03′W | 6.0 | 0.3 | 30 | 14.3±2.0 | 1.5±0.2 | 1.8±0.3 |
| Taylor | 45°36′N, 76°02′W | 4.0 | 0.3 | 70 | 15.9±2.1 | 1.7±0.5 | 3.0±0.5 |

**Note**: Values are given as the mean ± standard error. Chl *a*, chlorophyll *a*.

## Introduction

Size distributions of organisms are potentially useful metrics of the structure of natural communities because they do not require extensive taxonomic expertise, they simplify comparisons among communities formed of different taxa, and they can be combined with general allometric relationships to estimate community metabolism (Platt et al. 1984; Gaedke 1993). These desirable properties explain the increasing use of size-based descriptions of natural communities and their inclusion in theories of energy transfers in ecosystems. However, before size distributions can be incorporated in environmental assessment programs as diagnostic measures of community or ecosystem structure, it is necessary to quantify their seasonal variation and the effects of natural conditions on the size spectra.

To date, quantitative analyses of benthic size spectra have generally relied on two approaches: linear regression analyses of the normalized size spectrum (e.g., Hanson et al. 1989; Hanson 1990; Rodríguez and Magnan 1993; Rasmussen 1993), and multivariate analyses with abundance or biomass in each size-class considered as a variable (Rodríguez and Magnan 1993; Rasmussen 1993). The regression approach has two serious drawbacks. First, in contrast with pelagic communities, reported size distributions for the benthos usually are nonuniform across size-classes (Schwinghamer 1981; Strayer 1986; Hanson et al. 1989; Hanson 1990; Morin and Nadon 1991; Strayer 1991; Rodríguez and Magnan 1993; Rasmussen 1993). Consequently, benthic normalized spectra often are strongly nonlinear and have peaks and troughs that reflect the presence of one or more modes in the size distribution. Second, because adjacent size-classes tend to vary together, the regression residuals usually are strongly autocorrelated, which impairs statistical inferences that compare regression slopes or relate the slopes to environmental variables. Although multivariate techniques such as canonical correspondence analysis (Rodríguez and Magnan 1993) and principal components analysis (Rasmussen 1993) take into account the correlations between different size-classes, they do not exploit the information inherent in the size ordering of variables, i.e., they do not a priori distinguish between two variables representing adjacent size-classes and a third one representing a much larger, or much smaller, size-class.

Two multivariate techniques that have been used for studying shape variation in biological specimens, relative warp analysis (RWA) and thin-plate splines (TPS) (Bookstein 1991; Rohlf 1993), overcome the difficulties enumerated above. RWA and TPS allow one to decompose the shape variation among objects within a sample into a uniform component, corresponding to linear scale changes along the *x* and *y* axes, and a nonuniform component, corresponding to localized shape deformations. These techniques take into account the correlations between size-class abundances and the ordering of size-classes, and they allow for direct visualization of correlated changes in abundance.

Here, we describe the variability of the size distribution of invertebrates on rocks in 12 streams covering a range of trophic conditions and human impact during a 15-month period. We then use RWA and TPS to examine the major patterns of variation in the normalized size spectrum of benthic stream invertebrates. We describe this variation in terms of uniform and nonuniform components that are displayed graphically, and relate those components to environmental variables. Finally, we describe the temporal pattern of total community production and discuss the relative contribution of organisms of various sizes to total production.

## Methods and materials

### Study area

We examined three groups of four streams of the Ottawa–Hull area selected to cover the local range of trophic conditions in urban, forested, and protected land areas (Table 1). The four urban sites (Des Trembles, Green, Leamy, and Sawmill) were located within the boundaries of the municipalities of Ottawa–Carleton and Hull. The four Outaouais sites (Blackburn, Corriveau, Pélissier, and Rainville) were on the Quebec side of the Ottawa Valley in a forested cottage area. The remaining sites (Chelsea, Lapêche Sud, Renaud, and Taylor) drained protected forested areas in Gatineau Park. All sampling was done at a single location in each stream: a shallow riffle with substrate dominated by cobbles and rocks of 4–20 cm diameter.

### Sampling and laboratory methods

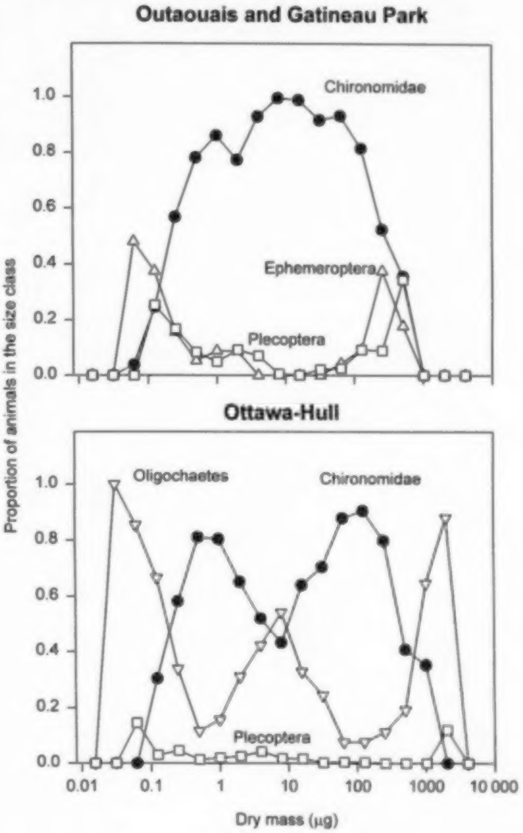
Each stream was sampled 14 times between May 1990 and August 1991 at approximately biweekly intervals in spring and summer and every 6–7 weeks the rest of the year. At each sampling occasion, we measured water temperature and collected eight rocks at random locations within the riffle (average total surface area of rocks = 105 cm$^2$, range = 20–350 cm$^2$) by gently lifting them from the stream bottom and inserting them in plastic bags. A measured volume of 95% ethanol was then poured in the bag to entirely immerse the rock and preserve the sample.

At each sampling site, three 4-L bottles were filled with water by immersion. To determine the sestonic chlorophyll *a* concentration, we filtered 500 mL of water, put the filter in 12 mL of 95% ethanol, and used the method of Ostrofsky and Rigler (1987). Total seston concentration was estimated from the dry mass retained on a Gelman A/E glass fiber filter. Total phosphorus concentration was estimated after potassium persulphate digestion under pressure (Menzel and Corwin 1965).

Periphyton biomass was estimated from the amount of chlorophyll *a* extracted by the ethanol used to preserve the benthos samples. A 12-mL aliquot of ethanol was taken from each sampling bag 24 h after the collection. Chlorophyll *a* concentration was estimated spectrophotometrically by the method of Ostrofsky and Rigler (1987). Periphyton biomass was expressed in milligrams of chlorophyll *a* per square metre of rock surface. The surface area of rocks was estimated by measuring the mass of aluminium foil necessary to wrap them, and converting the mass into surface area using a calibration curve.

Benthos samples were sorted under a dissecting microscope at 25×. When they contained more than 200 individuals, they were separated into two fractions (>1 mm, 63 μm to 1 mm). The animals in the >1 mm fraction were all sorted whereas the ones in the 63 μm – 1 mm fraction were subsampled with a Folsom plankton splitter until 50–200 individuals remained. The invertebrates were then measured to the nearest 0.01 mm using an image analysis system. The individual dry mass (DM, in μg) was estimated by the equation DM = $L^3$, where $L$ is body length (mm) (Morin and Nadon 1991). Density and biomass were obtained by dividing the number of animals or their total dry mass by the rock surface area.

**Fig. 1.** Average proportion of the most abundant taxa in each size-class for sites in cottage areas and protected parkland (Outaouais and Gatineau Park) and in urban sites (Ottawa–Hull). Note the wide size range covered by each taxon.

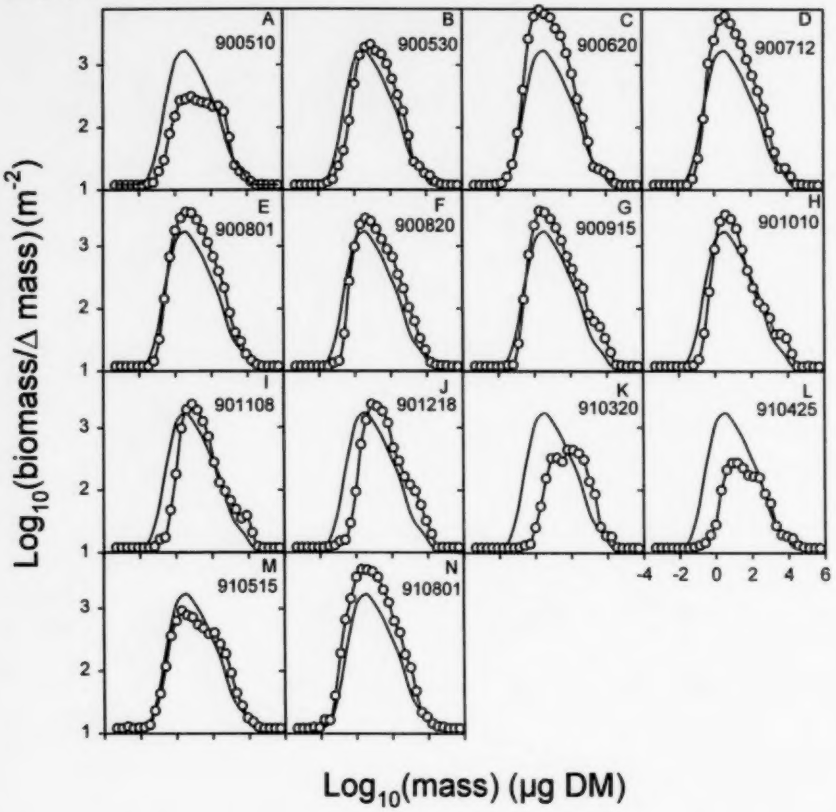


The size distribution of organisms in each site on each sample date was quantified by calculating the average normalized spectra from the eight replicates. Collected organisms were first assigned to one of 31 logarithmic size-classes ranging from $2^{-12}$ to $2^{18}$ μg. The total biomass in each size-class (μg DM·m$^{-2}$) was then divided by the width of the size-class (μg DM) to obtain the normalized biomass (m$^{-2}$). Then we calculated the average of the log of the normalized biomasses in the replicate samples. Because several size-classes contained no individuals, we added a constant corresponding to the detection limit to the normalized biomass before log transforming the data. The detection limit was calculated as 12 m$^{-2}$, corresponding to only one individual in the eight replicate samples (averaging 840 cm$^2$).

### Relative warp analysis

RWA (Bookstein 1989, 1991; Rohlf 1993) is a multivariate statistical technique that allows one to succinctly describe

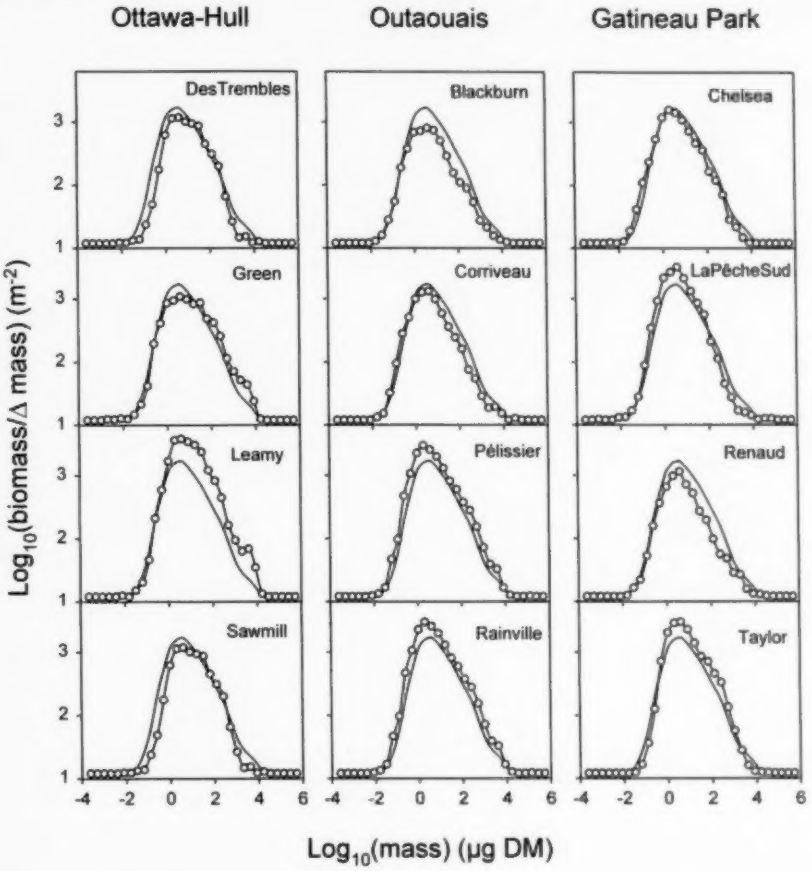
**Fig. 2.** Normalized biomass spectra averaged across sites on each of 14 sampling dates (YYMMDD). The recurring curve on each panel is the average of all spectra used as the reference in the analysis.



the patterns of shape variation among objects within a collection. The point of departure in RWA is the construction of a reference configuration that represents a typical or average object in the collection. Each of the *n* objects is characterized by the coordinates of a set of *K* points, or landmarks, which are distributed over the outline or the surface of the object and which ideally capture all of the shape features of interest. The reference configuration can be obtained, for instance, by calculating the mean of the *x*, *y* coordinates at each point location. Next, an eigenanalysis based on the reference configuration coordinates is conducted to calculate *K*-3 principal warps, eigenvectors that are solely a function of the reference configuration. The principal warps define a space that furnishes a geometrically convenient framework for describing nonuniform shape changes. Principal warps are ordered according to the magnitude of their associated eigenvalues, which are inversely related to spatial scale; those warps with large associated eigenvalues describe small-scale shape changes (pertaining to neighbouring landmarks) whereas those with small associated eigenvalues describe large-scale shape changes (distributed across many landmark points). The eigenanalysis that yields the principal warps separates the uniform component of shape variation from the nonuniform component, because there are three trivial principal warps with zero eigenvalues, corresponding to uniform transformations that have infinite spatial scale (simple rotation, translation, and dilation of the object). Finally, the deviations of the *n* objects from the reference configuration at each landmark point are scaled and projected onto the principal warps, and the resulting matrix is factored to extract its principal components, the relative warps. The relative warps are eigenvectors depicting the patterns of nonuniform shape variation within the collection of objects, with the first relative warp accounting for the largest portion of this variation and each subsequent warp accounting for progressively less variation. This feature may allow one to retain for further analysis only a few higher order warps that account for a substantial portion of the variation in nonuniform shape. Unlike the principal warps, the relative warps are influenced by the variation among objects in the sample. For interpretative purposes, the loadings of the principal warps on the relative warps can be examined to determine which principal warps are most strongly related to a given relative warp.

As in more conventional applications of principal components analysis, the scores of objects on the relative warps are uncorrelated and can be used to plot the objects in an

**Fig. 3.** Normalized biomass spectra averaged across sampling dates at each site. The recurring curve on each panel is the average of all spectra used as the reference in the analysis.

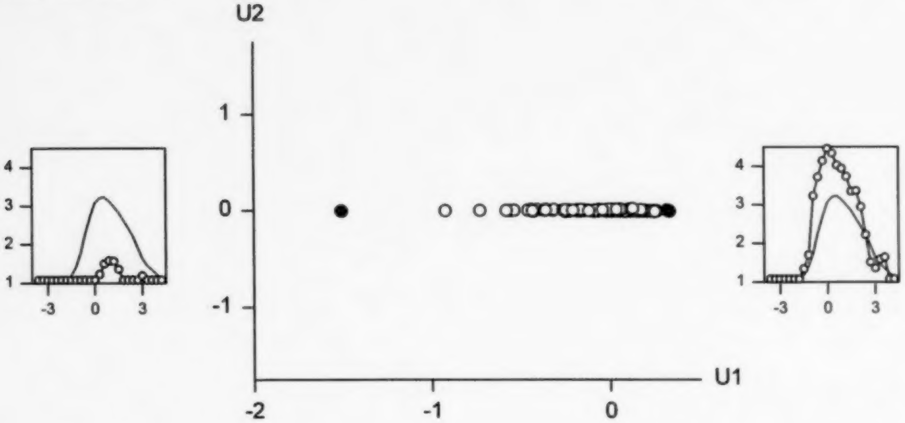


ordination diagram in which objects with similar shape (after extraction of the uniform component) should be close together. The shape change associated with each relative warp can be displayed effectively as deformations of a regular orthogonal grid containing the reference object. For each relative warp, a TPS function is derived that describes the deformations associated with displacements along the warp. The reference configuration, which corresponds to a value of zero on the relative warp, is then presented alongside two deformed grids, one corresponding to a positive value along a selected relative warp, and another corresponding to a negative value.

In the present study, the objects of interest are the configurations of points given by the *x*, *y* coordinates of the 168 normalized size spectra. The *x* coordinates specify the upper limits of 31 size-classes (μg) on a $\log_{10}$ scale, and are identical for all samples. The *y* coordinates are the normalized biomass (the numerical density of individuals in each size-class times the geometric mean weight for the size-class, divided by the width of the size-class) on a $\log_{10}$ scale. Therefore, the *y* values are directly proportional to $\log_{10}$(numerical density). The reference configuration, or typical spectrum, is given by the means of the *x*, *y* coordinates. All spectra, including the reference spectrum, share at least two points $((x_{\min}, y_{\min})$ and $(x_{\max}, y_{\min}))$ that anchor a common baseline. Alignment to this baseline provides a natural scaling for the spectra and excludes differences owing to rotation and translation.

The deviations of individual spectra from the reference configuration are shape changes having uniform and nonuniform components. The uniform component has two parts: dilation or contraction parallel to the *x* axis, and vertical dilation and contraction along the *y* axis. The latter part can be interpreted as a generalized measure of numerical density (on a $\log_{10}$ scale), smoothed across size-classes. The nonuniform component of shape variation in the spectrum (i.e., localized change in the configuration of points) describes changes in the relative abundance of size-classes at different scales, e.g., increase in the density of the 15 smallest size-classes relative to the 15 largest size-classes (a large-scale change), or increase in the density of a single size-class relative to all others (a small-scale change).

**Fig. 4.** Ordination diagram of the uniform component of shape variation among the 168 size spectra. The first uniform component can be interpreted as a generalized index of abundance over all size-classes. The smaller graphics show the normalized spectra (connected dots) for two extreme samples having the lowest and the highest values along the horizontal axis of the ordination diagram (solid symbols). The reference configuration (solid curve) is included for comparison.



We used the thin-plate splines relative warp analysis program (TPSRW; 2 October 1993 version, written by F.J. Rohlf) to decompose the spectral shape variation into its uniform and nonuniform components and to calculate TPS for graphical display. The extremities of the normalized spectra were used to provide a baseline for calculation of shape coordinates (Bookstein coordinates option in TPSRW). The uniform component was calculated as the *x,y* projections of each sample from the polar diagram (X option in TPSRW). The α parameter, which determines how the principal warps are weighted in the analysis, was set to zero. This choice weighs all principal warps equally, which ensures that no a priori preference is given to changes at particular scales.

Patterns of variation of the uniform and nonuniform components of spectral shape were examined by analysis of covariance (ANCOVA). Sample scores on each component were regressed against water temperature, periphyton biomass, and total phosphorus concentration in the water and compared among the three groups of sites. This analysis allowed for the comparison of spectra, corrected for environmental variables included in the ANCOVA model, among the three groups of sites.

### Community production

Total community production was estimated by combining the average size distribution observed on each date to an empirical model of instantaneous growth rate of lotic insects as a function of individual size and water temperature (Morin and Dumont 1994). Normalized biomass in each size-class was multiplied by the predicted growth rate for animals of a size equal to the geometric average of the limits of the size-class at the average temperature observed on that sampling date. Production values of all size-classes were then summed to estimate total community production.

## Results

### Composition of sampled communities

Although chironomids were dominant in all sites, there was a clear difference in community composition between sites. Larval chironomids accounted for between 60 and 94% of the organisms collected on all date–site combinations. Their relative abundance, however, varied systematically between the three groups of sites. They were less dominant in city sites (68%, SE = 6%, $n = 4$) where oligochaetes (27%, SE = 3%, $n = 4$) were the codominant taxa, than in the two other groups of sites where they averaged 83% (SE = 4%, $n = 8$) of all invertebrates collected.

The taxonomic composition of groups of animals of similar size also differed among the three groups of sites (Fig. 1). In the Outaouais and Gatineau Park streams, chironomids dominated all size-classes between 0.25 and 128 μg DM and codominated with plecopterans and ephemeropterans the size-classes smaller than 0.25 μg and those larger than 128 μg. In the urban sites of Ottawa–Hull, oligochaetes were dominant in the smallest and largest size-classes, and codominant in the 2–32 μg size range. Chironomids clearly dominated only the 0.25–2 and 32–256 μg size range.

### Seasonal patterns and differences among sites

The shape of the average size spectrum of all sites varied mainly in height among dates (Fig. 2). Numerical abundance, proportional to the normalized biomass, peaked in June and July, and reached a minimum in late April and early May, just after spring runoff. Temporal variation in abundance was larger for animals of intermediate sizes (approximately from 1 to 125 μg, equivalent to 1 to 5 mm), which are mainly larval chironomids. Average size varied throughout the year, reaching a minimum in June (11 μg) and a maximum in late winter (150 μg).

**Table 2.** Loadings of the principal warps in the *y* direction on the first five relative warps, and Pearson correlation of the principal warps with water temperature (TEMP), log periphyton chlorophyll *a* concentration (LPERI, $mg \cdot m^{-2}$) and log total phosphorus concentration (LTP, $\mu g \cdot L^{-1}$).

| Principal warp | Eigenvalue | Relative warp loading | | | | | Correlation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RW1 | RW2 | RW3 | RW4 | RW5 | TEMP | LPERI | LTP |
| 1 | 944.4 | 0 | −0.01 | −0.01 | −0.01 | 0.01 | 0.1 | 0.12 | 0.11 |
| 2 | 931.1 | 0 | 0 | 0.02 | −0.03 | −0.02 | −0.06 | −0.04 | 0.04 |
| 3 | 815.3 | 0.01 | 0.01 | 0 | 0.01 | −0.02 | 0.07 | −0.16 | −0.14 |
| 4 | 768.4 | 0 | 0 | −0.01 | 0.02 | 0 | −0.05 | 0.14 | 0.06 |
| 5 | 691.6 | 0 | 0 | 0.01 | −0.01 | 0 | −0.06 | −0.06 | −0.07 |
| 6 | 647.0 | 0 | 0.02 | 0 | 0 | −0.01 | 0.02 | −0.04 | 0.01 |
| 7 | 591.9 | 0.02 | 0.01 | −0.02 | 0.06 | 0.08 | 0.24 | −0.23 | −0.21 |
| 8 | 514.1 | 0 | 0.01 | −0.01 | −0.01 | 0.05 | 0.04 | 0.05 | −0.01 |
| 9 | 448.4 | 0.01 | −0.01 | 0.01 | −0.04 | 0.02 | 0.02 | 0.05 | −0.03 |
| 10 | 427.2 | −0.03 | −0.02 | 0.01 | −0.11 | −0.09 | −0.19 | 0.09 | 0.26* |
| 11 | 358.6 | 0.01 | 0.03 | 0 | −0.01 | 0.01 | 0 | 0.09 | −0.05 |
| 12 | 299.6 | 0.01 | 0.02 | −0.03 | 0.01 | 0.06 | −0.04 | −0.04 | −0.05 |
| 13 | 273.9 | 0 | −0.02 | 0.01 | −0.06 | 0.04 | −0.08 | 0.18 | 0.08 |
| 14 | 236.1 | 0.01 | 0 | −0.09 | −0.08 | 0.11 | 0 | −0.09 | −0.07 |
| 15 | 200.1 | −0.04 | −0.01 | −0.01 | −0.04 | 0.04 | −0.11 | 0.04 | 0.13 |
| 16 | 162.4 | 0 | −0.01 | −0.03 | −0.06 | 0.06 | −0.02 | 0.06 | 0.10 |
| 17 | 136.7 | −0.01 | −0.02 | −0.04 | −0.04 | 0.05 | 0 | −0.18 | −0.08 |
| 18 | 108.5 | 0.01 | 0.01 | −0.13 | −0.13 | 0.24 | −0.03 | −0.1 | −0.07 |
| 19 | 84.6 | 0.01 | 0.01 | −0.02 | −0.09 | −0.11 | −0.12 | 0.06 | 0.06 |
| 20 | 64.8 | 0 | 0.12 | 0.13 | 0.03 | −0.24 | −0.09 | 0.1 | 0.05 |
| 21 | 49.5 | 0.04 | −0.03 | 0.1 | −0.31 | 0.19 | 0.02 | −0.06 | −0.17 |
| 22 | 35.0 | −0.09 | −0.06 | −0.14 | −0.05 | 0.75 | −0.28* | −0.18 | −0.04 |
| 23 | 25.0 | −0.04 | −0.12 | −0.08 | 0.77 | 0.12 | 0.22 | −0.16 | −0.08 |
| 24 | 15.6 | −0.2 | 0.28 | 0.53 | 0.02 | 0.42 | −0.55* | 0.02 | 0.13 |
| 25 | 10.0 | −0.28 | 0.6 | −0.3 | −0.3 | −0.1 | −0.49* | 0.02 | 0.24 |
| 26 | 4.6 | 0.4 | 0.34 | 0.62 | 0.06 | −0.03 | 0.29* | 0.11 | −0.21 |
| 27 | 2.0 | 0.67 | −0.31 | −0.05 | −0.29 | 0.1 | 0.55* | 0.04 | −0.28* |
| 28 | 1.1 | −0.52 | −0.56 | 0.4 | −0.25 | −0.13 | −0.43* | 0.02 | 0.28* |
| % variation accounted for by each relative warp | | 21.8 | 12.4 | 9.1 | 7.6 | 6.2 | | | |
| Cumulative % | | 21.8 | 34.2 | 43.3 | 50.9 | 57.1 | | | |

**Note**: Large eigenvalues correspond to small-scale deformations that reflect changes in one or a few size-classes, and small eigenvalues correspond to large-scale changes distributed among many size-classes. Loadings in the *x* direction are zero because there is no among-sample variation along the *x* axis.

*Significant at the 5% level, corrected for the number of correlations (Bonferroni correction).

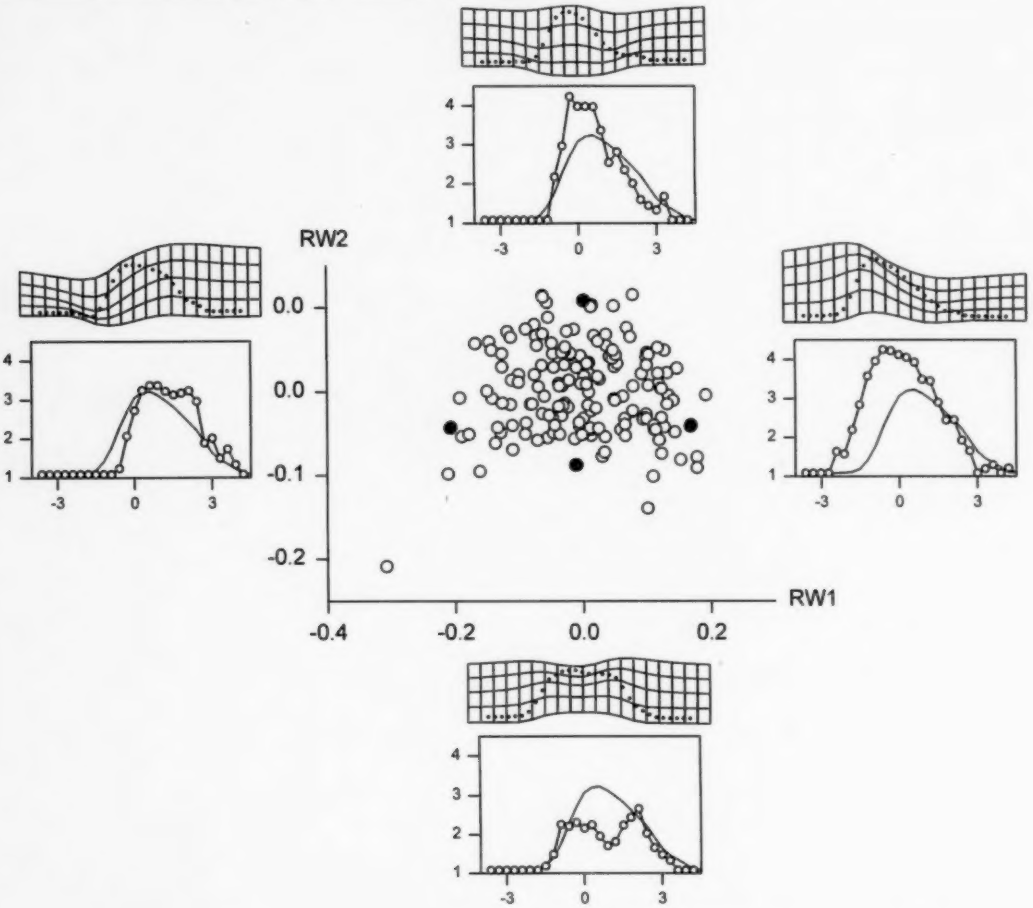
The annual average size spectrum was very similar among the 12 sites (Fig. 3). Again, the unimodal distribution varied mainly in height among sites for animals in the 1–5 mm range. Average size varied little among sites, but was slightly higher in Ottawa–Hull sites (55 μg) than in streams from the Outaouais and Gatineau Park (34 μg).

### Relative warp analysis

The uniform component of shape variation among the 168 samples was essentially one-dimensional and was closely aligned with the horizontal axis of the ordination diagram (Fig. 4). This result indicates that samples differed in the uniform component mostly by stretching or contraction along the *y* axis of the normalized spectra, reflecting differences in overall abundance. The sample scores along the horizontal axis of the ordination, which estimate the extent of uniform stretching or contraction along the *y* axis, correlated highly ($r = 0.75$) with the average *y* values calculated across size-classes for each sample, which provide an independent measure of overall abundance. Very little lateral shear was evident in the ordination, as shown by the restricted range of sample scores along the vertical axis (Fig. 4).

The first five relative warps accounted for 57.1% of the nonuniform component (Table 2). The pattern of decay in the proportion of variation accounted for by successive relative warps (a scree plot, not presented here) suggested that only five warps should be retained for further analysis. The highest relative warp loadings (Table 2) corresponded to principal warps with small eigenvalues, indicating that

**Fig. 5.** Ordination of samples along the first two relative warps describing variation in shape of the 168 size spectra that is independent of overall abundance. The smaller graphics show the normalized spectra for two samples with extreme scores on relative warp 1 and near-average scores on relative warp 2, and for two samples with extreme scores on relative warp 2 and near-average scores on relative warp 1. These four samples are indicated by filled symbols on the ordination diagram. The reference configuration (solid curve) is included for comparison. Above each graph is the thin-plate spline representation of the nonuniform component of shape variation along the first two relative warps. The first relative warp axis (RW1) separates samples containing relatively few small animals from those having more small animals than average. Average size is therefore negatively correlated to sample score on RW1. The second relative warp axis (RW2) separates samples containing more intermediate sized animals than average (spectrum is sharply peaked) from those containing fewer animals of intermediate size than average (spectra that have a flatter top). Variance in individual size is therefore negatively correlated to sample score on RW2.



the major patterns of nonuniform variation in the spectra arose from large-scale changes distributed among many size-classes. These patterns are immediately visible in the TPS displays for the first two relative warps (Fig. 5). The first relative warp (21.8% of the nonuniform variation) depicted changes in the abundance of small organisms (approximately 0.05–2 μg, or 0.4–1.3 mm) relative to that of large ones (approximately 10 – 10 000 μg, or 2–20 mm). The second relative warp (12.4% of the nonuniform variation) contrasted flat-topped spectra, at one extreme, with peaked spectra, at the other (Fig. 5). Spectra with an extreme score along one of the warps and a near-average score along the other provide specific examples of these shape deformations (Fig. 5).

### Spectral variation in relation to temperature, algal abundance, total phosphorus, and season

Temperature was most strongly correlated with the size and shape of the spectra, explaining a significant portion of the variability of both uniform and nonuniform components

**Table 3.** Percent variation in spectral shape accounted for by linear trends with temperature, log periphyton chlorophyll *a* concentration (LPERI), and log total phosphorus concentration (LTP) in the water.

| Component | Temperature | LPERI | LTP |
|---|---|---|---|
| Uniform | | | |
| U1 | 7.3*** | 7.9*** | 1 |
| U2 | 6.9*** | 0.2 | 0.3 |
| Nonuniform | | | |
| RW1 | 37.6*** | 1.9 | 9.4** |
| RW2 | 5.1** | 2.3 | 0.1 |
| RW3 | 0.9 | 2.9* | 0.1 |
| RW4 | 3.3* | 0.1 | 1.1 |
| RW5 | 2.1 | 3.7* | 3.7* |

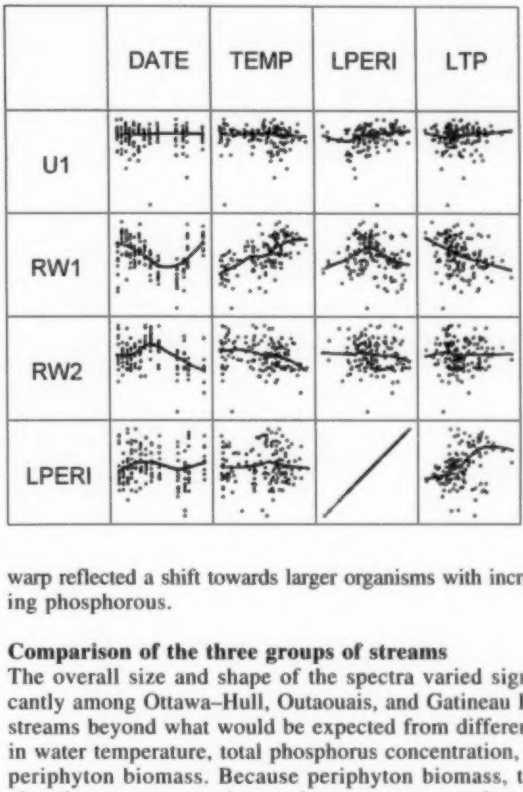
**Note**: The asterisks indicate the probability that the correlation between the component and the independent variable is zero (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

(Table 3). The positive relation between U1 and temperature reflects the increased overall abundance observed in summer compared with colder periods. In addition, temperature was significantly related to the nonuniform component (multiple regression on all the principal warps: $R^2 = 0.60$, $p = 0.001$). Temperature correlated best with those principal warps having small eigenvalues and with the first relative warp (Tables 2 and 3), indicating that temperature variation is predominantly associated with large-scale spectral changes. Temperature was well predicted by a cosifunction of the day of the year (DOY) (temperature = $t_0 + A\cos(2\pi\ \mathrm{DOY}/365 + d_0)$ , where $t_0$, $A$, and $d_0$ are fitted constants; $r^2 = 0.89$), indicating that, in our samples, variation in temperature was primarily due to cyclical seasonal change. Because temperature was reliably predicted by DOY, the correlation of temperature with the first relative warp implied a cyclical yearly pattern of nonuniform change in the spectra that closely resembled the major pattern of nonuniform variation described by relative warp 1 (Fig. 5). Temperature changes generated a seesaw effect through which smaller organisms became more abundant relative to larger ones as temperature rose in the spring and summer, with a reverse trend starting after summer peak temperatures and continuing into the autumn and winter.

Periphyton biomass influenced mainly the overall abundance, but was poorly related to the nonuniform component of the spectra (Tables 2 and 3). Algal abundance was positively related to U1, reflecting higher overall abundance in samples rich in periphyton. The plot of the first relative warp against log periphyton biomass suggested a weak curvilinear trend (Fig. 6) that would correspond to a shift towards smaller organisms at intermediate values of periphyton biomass.

Total phosphorus concentration was not significantly related to the uniform components of spectral variation (Fig. 6, Table 3), but was significantly related to the nonuniform variation in spectral shape (multiple regression of log total phosphorus on all of the principal warps: $R^2 = 0.30$; $p = 0.01$). The negative correlation with the first relative warp reflected a shift towards larger organisms with increasing phosphorous.

**Fig. 6.** Shape change in the normalized spectra in relation to date and environmental variables. The scatterplots depict the first uniform component (U1), the first two relative warps (RW1 and RW2), and log periphyton chlorophyll *a* concentration (LPERI) as a function of date, temperature (TEMP), log periphyton chlorophyll *a* concentration, and log total phosphorus concentration in the water (LTP). The curves are robust locally weighted regressions (LOWESS with tension factor = 0.5; Cleveland 1985).

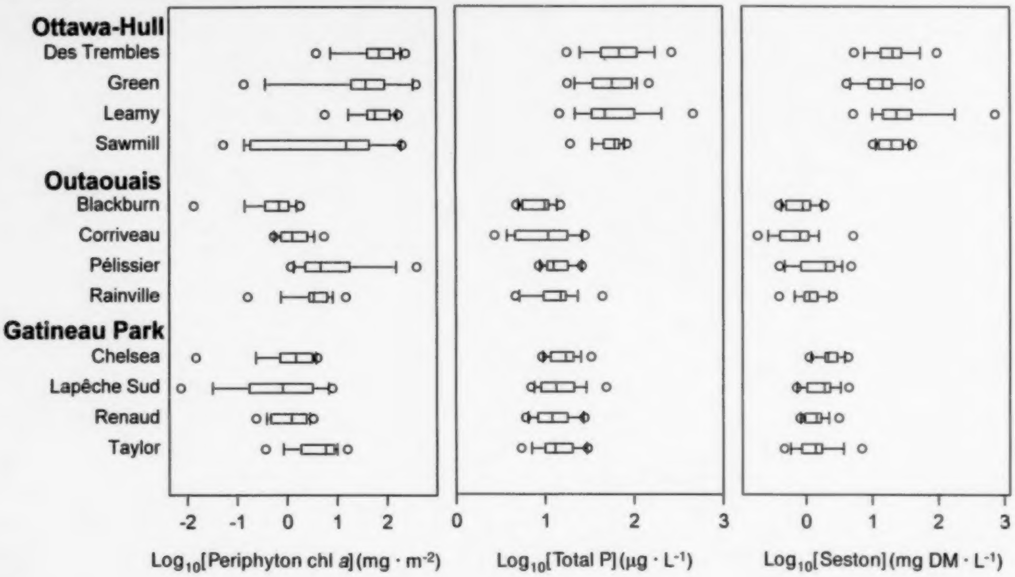


### Comparison of the three groups of streams

The overall size and shape of the spectra varied significantly among Ottawa–Hull, Outaouais, and Gatineau Park streams beyond what would be expected from differences in water temperature, total phosphorus concentration, and periphyton biomass. Because periphyton biomass, total phosphorus concentration, and seston concentration varied among the three groups (Fig. 7), it was necessary to account for these differences before comparing regions. The uniform component (U1) was positively related to log periphyton biomass ($p < 0.01$), and varied between the three groups of sites (ANCOVA with log periphyton chlorophyll *a* concentration, temperature, and log total phosphorus concentration as covariates, $p < 0.001$) but was not related to temperature and log phosphorus concentration. Sites from the Outaouais had an average score on this component that was higher than the scores for the other two groups (Bonferroni adjusted *t* test, $p < 0.01$), reflecting the high normalized biomass in intermediate size-classes in Pélissier and Rainville streams (Fig. 3).

The first relative warp (RW1), which reflects average size, was positively related to temperature ($p < 0.001$), was unrelated to periphyton biomass and phosphorus concentration (both $p > 0.7$), and varied among the three regions (ANCOVA, $p = 0.001$). Ottawa–Hull sites had

**Fig. 7.** Box-and-whiskers plots illustrating the temporal variation in periphyton chlorophyll, total phosphorus, and seston concentration readings for each site. The median is given by the vertical line within the boxes and the interquartile range is given by the left and right limits of the boxes. The horizontal lines (whiskers) show the range of values falling within the 10 and 90% percentiles. Circles represent outliers.



a lower adjusted RW1 score (Bonferroni adjusted *t* test, $p < 0.001$) than sites from the two other groups, reflecting an increase in average individual size in the city sites.

### Community production

Average total community production fluctuated seasonally, peaking in early summer and reaching a minimum in winter when water temperatures reached their minimum (Fig. 8). Most of the production resulted from the growth of animals between 1 and 10 mm long, their contribution averaging 80% of total production. On average, animals smaller than 1 mm contributed 3% of total production, whereas animals larger than 10 mm contributed 17%. The relative contribution of these three size categories varied seasonally. The contribution of the smallest organisms peaked in early summer, whereas that of the largest ones reached its maximum in the fall and late spring.

## Discussion

### Temporal variability in size spectra and community production

Although the size distribution of invertebrates varied statistically through time, the most striking feature remains the similarity of average size distributions among dates (Fig. 2). Size distributions in our streams appear to be remarkably stable despite considerable changes in species composition throughout the year. The continuous recruitment of early instars of various insect species during spring, summer, and early fall contributes to maintaining the shape of the overall distribution despite growth and emergence.

The similarity of invertebrate size distributions in our streams throughout the summer contrasts with what has been reported for lake littoral communities, but this may be due to a difference in the way results are reported. Hanson et al. (1989) concluded that there were strong seasonal patterns in the biomass size distributions of invertebrates of an Alberta lake and attributed these variations to the phenology of the dominant species. Their conclusion was based on a series of histograms of arithmetic mean biomass in each size-class for each sampling date that showed the appearance and disappearance of several modes in the biomass size distribution. Our analysis is based on log transformed data that are less affected by extreme values, and twofold variations that would appear as obvious deviations from the average pattern if data were presented on an arithmetic scale are made much less conspicuous in our spectra. Indeed, a reanalysis of the data of Hanson et al. (1989) for the littoral zone suggests that temporal variability in normalized biomass is larger in our streams than in the lake they studied. After conversion of their data to a normalized size spectrum, we computed a two-way analysis of variance (ANOVA) of the log(biomass/$\Delta$mass) on size-classes and sampling dates to estimate the temporal variability from the mean square associated with sampling date. Similar ANOVAs were then computed on summer data for each of the streams. On average, the temporal variability in our streams was an order of magnitude larger than what we computed for the lake data. Thus, it appears that size spectra in streams, even if they remain similar in shape throughout the summer, are more variable than those in lakes.

The temporal changes in the overall abundance and size distribution of the benthic community reflect the life history of the dominant group present in our streams. The most abundant and temporally variable small invertebrates were dominated by chironomid larvae that hatch in high numbers in late May and remain abundant during the summer, but become less abundant when water temperature drops below 10°C in the fall. Their relative abundance peaked in June when total community production reached its maximum. Although these smallest invertebrates were numerous, their average contribution to community production never reached more than one fifth of total production (Fig. 8).

The strong correlation between total community production and water temperature is not surprising, since the growth rates of lotic insects almost double for each increase in water temperature of 10°C. The fourfold difference in total production between summer and winter thus corresponds closely to what would be expected if abundance and size distribution remained the same while water temperature changed by 20°C. Total community production is therefore more a function of total biomass and water temperature than of the taxonomic composition and size distribution of the community.

The relatively small contribution of the smallest invertebrates to total community production confirms the speculation of Morin and Nadon (1991) that meiofaunal-sized organisms contribute little to epifaunal community metabolism on coarse substrates. In contrast, Poff et al. (1993) found that meiofauna in a sandy reach of Goose Creek accounted for 45% of community respiration estimated in summer at a temperature of 26°C, about 5°C higher than our warmest temperature. This high meiofaunal contribution may result from differences in substrate, or methodology, or from differences in environmental conditions between Goose Creek and our streams. Nevertheless, it fits the trend we observed of a higher contribution of small invertebrates to total community metabolism in warmer waters.

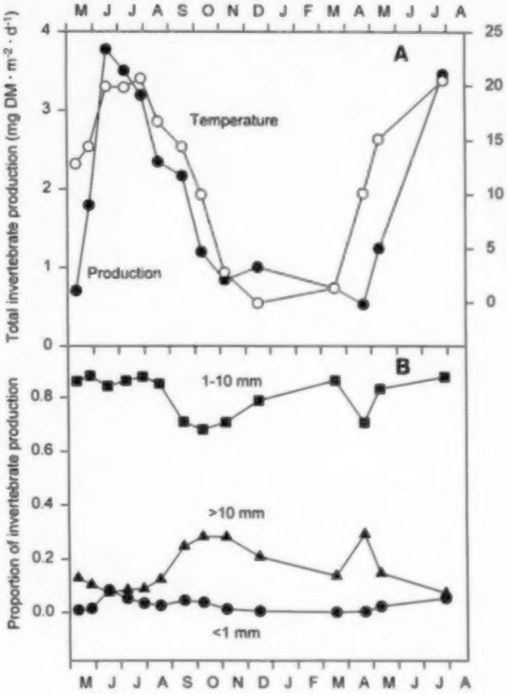
Because of the variability in both the overall abundance and the shape of the size distribution, sites or habitats should be compared for equivalent periods. Samples collected on different dates or at different water temperatures should not be directly compared until the seasonal and (or) temperature effects are accounted for.

### Intersite variation

Size spectra on rocks were remarkably similar among sites (Fig. 3), despite significant differences in both overall abundance and community composition. This regularity could be interpreted as evidence for an ecological constraint on size distributions in these habitats, although it is unclear what mechanism could exert such a constraint. Hanson (1990) suggested that differences in substrate and habitat complexity could explain shifts in the size distribution of invertebrates in *Chara* beds versus what he observed in other weed beds by affecting the efficiency of fish predators to capture invertebrates. However, examination of size distributions on substrates ranging from sand to boulders in a subset of our streams revealed that size spectra were very insensitive to changes in substrates (Nathalie Bourassa, University of Ottawa, personal communication). The putative mechanism constraining the general shape of spectra thus appears to be independent of season, trophy, and substrate.

The positive correlation between the first uniform component, a generalized index of abundance in all size-classes, and periphyton biomass suggests that the community biomass of secondary producers may be limited by the biomass of primary producers. Although the principal sources of organic matter to streams are allochtonous in forested areas (Vannote et al. 1980), autochtonous production may be more easily assimilated by invertebrates (Minshall 1978), leading to the observed weak correlations between the generalized index of abundance and periphyton standing stocks.

**Fig. 8.** Seasonal patterns of total community production (A) and contribution of three size categories to total production (B). Community production was estimated from the average size distribution and water temperature observed on each sampling date and the model of Morin and Dumont (1994) predicting instantaneous growth rates from body mass and water temperature.



It is unclear why the average size of invertebrates (as reflected by the RW1 component) is larger in city sites. These sites have higher phosphorus, seston, and periphyton standing stock than sites in the two other categories. They are also more affected by human activities and probably have higher levels of pollutants and pesticides. Although we attempted to account for the effect of trophy by correcting for periphyton standing stock and phosphorus in our analysis of covariance, we recognize that these indirect measures of food availability are imperfect. It is therefore possible that the larger average size is more an effect of food availability than of human impacts. The value of size structure as a community index of pollution remains to be examined in situations where pollution, for example by metals or pesticides, is not correlated with trophy.

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# Ecological uses for genetic algorithms: predicting fish distributions in complex physical habitats

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**Abstract**: Genetic algorithms (GA) are artificial intelligence techniques based on the theory of evolution that through the process of natural selection evolve formulae to solve problems or develop control strategies. We designed a GA to examine relationships between stream physical characteristics and trout distribution data for 3rd-, 5th-, and 7th-order stream sites in the Cascade Mountains, Oregon. Although traditional multivariate statistical techniques can perform this particular task, GAs are not constrained by assumptions of independence and linearity and therefore provide a useful alternative. To help gauge the effectiveness of the GA, we compared GA results with results from proportional trout distributions and multiple linear regression equations. The GA was a more effective predictor of trout distributions (paired $t$ test, $P < 0.05$) than other methods and also provided new insights into relationships between stream geomorphology and trout distributions. Most importantly, GA equations emphasized the nonindependence of stream channel units by revealing that (*i*) the factors that influence trout distributions change along a downstream continuum, and (*ii*) channel unit sequence can be critical. Superior performance of the GA, along with the new information it provided, indicates that genetic algorithms may provide a useful alternative or supportive method to statistical techniques.

**Résumé** : Les algorithmes génétique (AG) sont des techniques d'intelligence artificielle basées sur la théorie de l'évolution qui, par le processus de la sélection naturelle, élaborent des formules permettant de résoudre les problèmes ou de construire des stratégies de contrôle. Nous avons conçu un AG pour examiner les relations entre les caractéristiques physiques d'un cours d'eau et les données sur la répartition des truites à des sites situés dans des rivières de troisième, cinquième et septième ordre, dans les monts Cascade, en Oregon. Bien que les techniques statistiques multivariées traditionnelles puissent accomplir cette tâche particulière, les AG ne sont pas restreints par des hypothèses d'indépendance et de linéarité, et constituent donc une intéressante solution de rechange. Pour juger de l'efficacité de l'AG, nous avons comparé ses résultats à ceux obtenus par les distributions proportionnelles des truites, et par les équations de régression linéaire multiple. L'AG a prédit de façon plus efficace les distributions des truites (test $t$ jumelé, $P < 0,05$) que les autres méthodes, et a aussi donné de nouvelles perspectives sur les relations entre la géomorphologie du cours d'eau et la distribution des truites. Aspect plus important, les équations de l'AG ont souligné la non-indépendance des unités de lit de cours d'eau en révélant (*i*) que les facteurs qui influent sur la distribution des truites changent selon un continuum orienté vers l'aval et (*ii*) que la séquence des unités de lit peut jouer un rôle critique. La meilleure performance de l'AG, ainsi que les informations nouvelles qu'il a fournies, indique que les algorithmes génétiques peuvent constituer une solution de rechange intéressante ou une méthode de soutien pour les techniques statistiques.

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## Introduction

Considerable effort has been expended in attempts to relate the distribution of organisms to their physical environment (Krebs 1985). These efforts to understand organism–habitat relationships have become increasingly important as land-use practices that alter the physical environment accelerate. Commonly used techniques include linear regressions (e.g., Swanston et al. 1977; Bozek and Rahel 1991), multiple linear regressions (MLR) (e.g., Swanston et al. 1977; Binns and Eiserman 1979; Corkum 1991; Kilgour and Mackie 1991; Blumstein 1992), principal component analyses (e.g., Grossman et al. 1991; Jackson 1993), discriminant analyses (e.g., Legendre and Legendre 1983; Williams 1983), and canonical correlation (Grossman and Freeman 1987). Fish habitat preference and standing stocks have also been evaluated with habitat quality index models (Binns and Eiserman 1979) and instream flow incremental methodology (Bovee 1982). Each of these methods has contributed substantially to our understanding of organism–habitat relationships, but confidence in their results is often limited by the inability to meet assumptions such as normally distributed variables, independence of variables, and model linearity (Mathur et al. 1985; Parsons and Hubert 1988; Heggenes et al. 1991). Further, the need for subjective manipulation of the data (i.e., transformations, subjective inclusion of interaction terms) to meet assumptions can potentially bias results.

Weaknesses in available techniques have necessitated a search for alternative methods with which to evaluate relationships between organisms and a complex environment. These efforts have identified statistical techniques not constrained by assumptions of linearity, independence of variables, and statistically identical population distributions. For example, Borcard et al. (1992), noting the interaction of abiotic and biotic processes in space and time, used constrained and partial canonical ordination techniques, canonical correspondence analysis, and redundancy analysis to identify spatial, environmental, and undetermined variation in species abundance. Difficulties imposed by non-numeric, spatially correlated data can be accommodated by geostatistical techniques (Rossi et al. 1992), and distribution-free statistical methods are available for analysis of non-normal data distributions (Potvin and Roff 1993). Each of these newly identified techniques overcomes some limitations of basic parametric techniques and provides valid population statistics, formulae, and coefficients that reflect relationships between dependent and independent variables. However, use of these statistics requires an understanding of their often complicated premises and the consequences of violating those assumptions.

We propose genetic algorithms (GA) as a dynamic method for evaluating relationships between organisms and their environment. Because GAs do not use statistical procedures, they are not limited by statistical assumptions. GA performance is, however, strongly influenced by design decisions made by the programmer (see Genetic Algorithms section below). With a GA, the designer has more flexibility than is available with many other types of procedures. They can be modified to accommodate important characteristics of a system, explore alternative hypotheses, and elucidate underlying mechanisms simply by adding variables to the program or altering fitness criteria. GAs readily lend themselves to the exploration of relationships between input and output data (e.g., input = environmental variables; output = predicted distributions of organisms), the development of strategies to control behavior or movement (e.g., of robots), and the determination of optimal genetic strategies of populations exposed to different environmental conditions, optimal foraging strategies, behavioral trade-offs, coevolution of predators and competitors, and many other complex issues. Results from GAs can be coupled with statistical techniques to provide a more complete data analysis procedure. Perhaps the greatest asset of GAs, however, is that they can explore complex data and evaluate hypotheses to suggest mechanistic relationships in a manner similar to systems modeling but without explicit programming.

Previous studies have demonstrated the validity of GAs as a robust, efficient, and effective search procedure for function optimization and control applications (Goldberg 1989). This work has paved the way for the use of GAs for a wide variety of applications including models of consumer choice (Greene and Smith 1987), evolution of single-celled organisms (Rosenberg 1970*a*, 1970*b*), spatial and temporal food availability (Sannier and Goodman 1987), and many others. One of the most important advantages of GAs for these applications, that a priori understanding of system relationships is not required, was demonstrated by Koza (1992) when he reevaluated Roughgarden's (1979) study of optimal food foraging strategies of anolis lizards. Roughgarden's foraging model required insight into the functional form of the feeding strategy solution, whereas the GA developed a similar optimal foraging strategy (based on fitness criteria) without any a priori information about the functional form of the solution.

Our objective was to evaluate the ability of the GA to explore and discover relationships between physical characteristics of streams and trout distributions. To accomplish this, we compared the observed autumn distribution of adult cutthroat (*Oncorhynchus clarki*) and rainbow trout (*Oncorhynchus mykiss*) with the distribution predicted by surface area and volume-based distributions, multivariate statistical techniques, and the GA. We used data collected from field sites along a 3rd- through 7th-order gradient of the McKenzie River drainage basin in the Cascade Mountains of Oregon.

### Genetic algorithms

To thoroughly evaluate a problem or hypothesis, all possible solutions should be considered. The set of all possible solutions to a problem (or set of all possible relationships between organisms and their environment) can be depicted as a multidimensional solution space. Solution spaces may range from smooth functions that can be solved with calculus-based methods to complex, discontinuous surfaces without smooth slopes. Genetic algorithms (GAs) are computer solution search and problem-solving techniques that are especially well suited for exploring complex, discontinuous solution spaces. GAs are based on the principles of evolution by natural selection (Holland 1975). Through the process of natural selection, GAs evolve formulae to solve problems or discover relationships. Selection rules are designed by the programmer to govern the genetic direction that is taken to evolve solutions to problems. Rules may be defined on the basis of the programmer's conceptions about how a system operates (e.g., select organisms that are better at procuring food) or may be independent of internal system operation (e.g., optimize for correlation between observed and predicted organism distributions). Rules may be altered simply to produce a desired outcome (i.e., without any implied causality) or to evaluate multiple hypotheses about how a system operates.

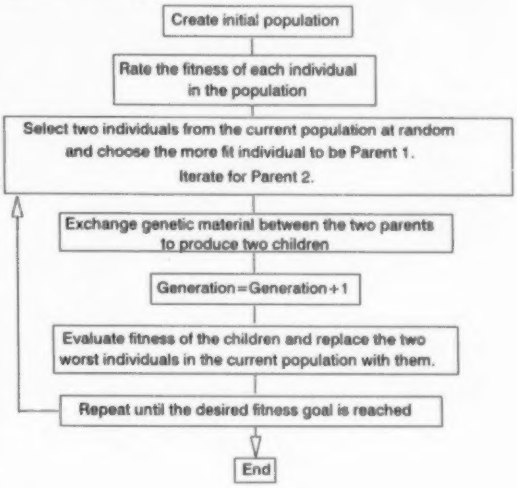
We describe the basic strategy for development of a GA and note the specific design decisions we chose for this application (Fig. 1). In general, GAs create a population of individuals. For our example, each individual in the population is represented by a formula comprised of two chromosomes, an equation chromosome and a coefficient chromosome, each with many genes. Genes on the equation chromosome represent a variable or mathematical operator of an equation to distribute fish. We included channel unit type, unit length, unit depth, reach type, pieces of wood, and substrate type as variables, and addition, subtraction, product, quotient, $x^y$, square root, and logarithm as mathematical operators. We limited the length of the formula, usually to less than 30 genes, to reduce the likelihood that an awkward, redundant equation would be produced. Genes on the coefficient chromosome represent coefficients for non-numeric variables (e.g., channel unit type) (Fig. 2). The coefficient chromosome includes a coefficient for each possible non-numeric variable that might occur on the equation chromosome. When a formula was evaluated the appropriate coefficient was substituted for the non-numeric variable. The chromosomes that make up individuals in the initial population can be created by a variety of methods. For this application, we randomly chose genes for the equation chromosomes from the pool of all possible variables and randomly chose coefficients for each gene on the coefficient chromosome. Individuals in the population (i.e., formulae made up by the equation and coefficient chromosomes) were then evaluated to determine their fitness (e.g., how well the formula predicted the distribution of fish).

Fitness evaluation is the most important determinant of how future recombinations are guided and the direction in which the population will evolve. The fitness of each formula supplies a point on the solution search space (i.e., set of all possible solutions). By creating a population of formulae, we can simultaneously evaluate multiple solutions in our quest to find a global optimum. For this GA, fitness was defined as the correlation between the number of fish predicted to be in a channel unit versus the actual number of fish in that channel unit. To calculate fitness of an individual formula, it must be evaluated at each stream channel unit. For example, if the formula for the first individual is

$$\text{No. of fish} = \text{coefficient } 1 \times \text{width} \times \text{depth}$$

where coefficient 1 = 0.5, width = 2 m, and depth = 2 m for the first channel unit, then individual one would predict two fish (i.e., $0.5 \times 2 \times 2$) to be in the first channel unit. This procedure is repeated for each channel unit and the fitness of the first individual is the resulting correlation between the number of fish predicted to be in each channel unit and the actual number of fish in each unit. Each individual formula is evaluated in this manner. Once fitness has been evaluated, individuals are chosen for reproduction using one of several selection techniques. Typically, those formulae with a high fitness value have a greater likelihood of being chosen as the two parents for reproduction.

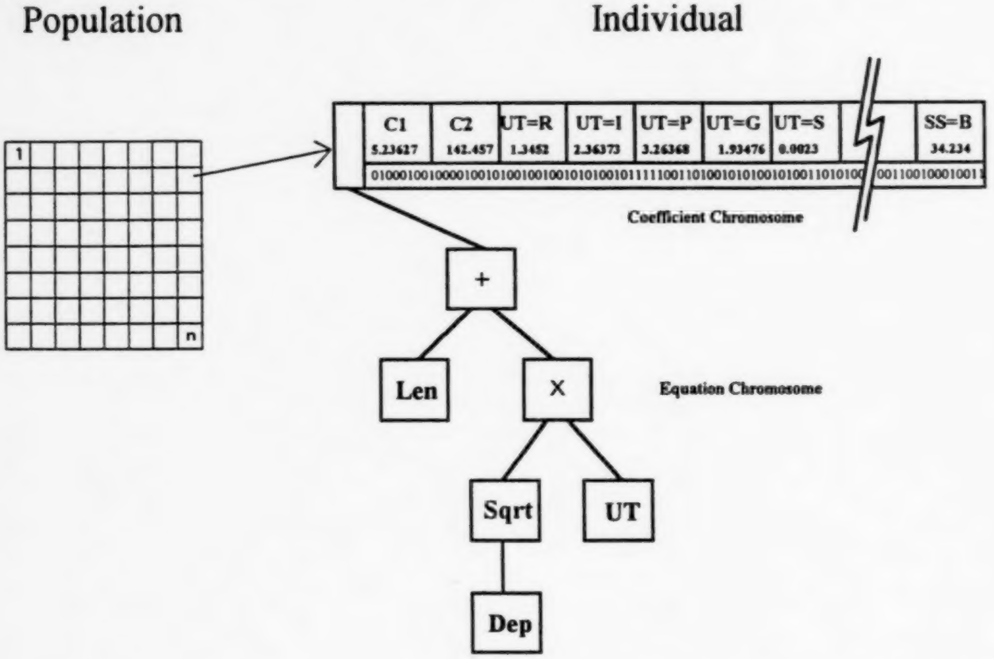
**Fig. 1.** Flow chart of genetic algorithm design and decision-making procedures.


Create initial population
Rate the fitness of each individual in the population
Select two individuals from the current population at random and choose the more fit individual to be Parent 1. Iterate for Parent 2.
Exchange genetic material between the two parents to produce two children
Generation = Generation + 1
Evaluate fitness of the children and replace the two worst individuals in the current population with them.
Repeat until the desired fitness goal is reached
End

Reproduction of individuals in the population occurs by making a copy of the chromosomes (one coefficient chromosome and one equation chromosome) from two parent individuals. Copies are then altered through crossover and mutation to provide the mechanism for evolution of the two new formulae (i.e., offspring) (Fig. 3). We used crossover and mutation rates of 0.80 and 0.10, respectively. Crossover involves the exchange of material between the two copies. Mutation involves the rare change or modification of a gene (i.e., a variable or mathematical operator) to a new randomly chosen gene with unpredictable consequences. Because offspring inherit both good and bad features of their parents and these features are recombined and altered through crossover and mutation, offspring may be more or less fit than their parents. After crossover and mutation, offspring may be added to the population or used to replace other less fit individuals (as in this model), or a whole generation of offspring may be created to replace the entire parent population. The GA is then run until formula improvements reach a desired goal. Our goal was to run the GA until performance increases reached a plateau. By conducting trial runs we determined that there was little improvement in performance after 20 000 generations. Because genetic algorithms have several random components, each run of the GA can produce a different equation and resultant distribution. There is no guarantee that a globally optimal solution will be obtained.

See Forrest (1993) for a brief but thorough introduction to GAs. Goldberg (1989) provides an easily understandable introductory text on GA development, while Koza (1992) supplies a more advanced treatment of GAs and an excellent reference text.

**Fig. 2.** Diagram of a population of *n* individuals with an example of an individual formula and associated coefficient strings. In the coefficient chromosome, C1 and C2 are generic coefficients, SS represents substrate types, and unit types (UTs) R, I, P, G, and S represent coefficients for the channel units rapids, riffles, pools, glides, and steps, respectively. In the equation chromosome, Len is the channel unit length and Dep is the channel unit depth. The remaining symbols in the formula are mathematical operators. The appropriate value for each variable is inserted into the formula from the coefficient string. For example, if the unit being evaluated with this formula was a pool then the number 3.263 68 would be inserted into the equation to replace UT.



## Methods

### Field sites

Field data were collected from three stream sites along a 3rd- through 7th-order gradient of the McKenzie River drainage basin in the Cascade Mountains of Oregon (Table 1, Fig. 4). Peak flows at these stream sites typically occur in late fall or early winter and low flows in late summer or early autumn. We sampled a 300-m section of Mack Creek, the 3rd-order stream site, in the center of the basin. The lower half of the study section in Mack Creek was logged in 1964 and has a canopy dominated by willow (*Salix* spp.). The upper half of the stream reach drains 400- to 500-year-old Douglas-fir (*Pseudotsuga menziesii*) forest with a much more extensive canopy than the clear-cut reach. Mack Creek flows into Lookout Creek, the 5th-order stream site, about 4.2 km downstream from the Mack Creek headwaters. We sampled a 1.5-km section of Lookout Creek near the mouth of the basin that drains the H.J. Andrews Long-term Ecological Research Site. Lookout Creek flows into Blue River and joins the McKenzie River 17.6 km from the Lookout Creek headwaters. We sampled a 22-km section of the South Fork McKenzie River (hereafter, called South Fork) above and below the Cougar Reservoir over an elevation range from 331 to 365 m. The river is impounded 7.8 km from the junction of the South Fork and main stem of the McKenzie River. The reservoir is approximately 125 m high and receives water from the upper 53 000 ha of the 138 000 ha basin, producing an 8–10 km long reservoir. The remaining 14.2-km section of the study reach was located above the reservoir. The reservoir was not sampled.

### Biological measurements

Survey crews also collected data on fish from all three stream sites during September and October 1987. To estimate cutthroat and rainbow trout abundance and distribution in Mack Creek, 50-m channel sections were blocked with nets at both ends and numbers of trout were estimated for each channel unit in the section by double-pass electroshocking (Armour et al. 1983). Trout numbers in Lookout Creek and South Fork were determined by snorkeling. Two to four divers swam upstream from the bottom of the unit to the top of the unit with one diver counting trout on the left half of the unit and one diver counting on the right half of the unit (Hankin and Reeves 1988). Fish were identified as either fry (0+ or young of the year) or adult (1+ and older). Only 1+ and older fish were used in these analyses. Fish that were removed from each channel unit by electroshocking were anesthetized, identified, weighed (wet

**Table 1.** Watershed and stream site characteristics for Mack Creek, Lookout Creek, and South Fork McKenzie River, Cascade Mountains, Oregon.

| | Mack Creek | Lookout Creek | South Fork |
|---|---|---|---|
| Order | 3 | 5 | 7 |
| Basin area (ha) | 640 | 6400 | 102 400 |
| Base elevation (m) | 655 | 412 | 365 |
| Peak elevation (m) | 1608 | 1630 | 3 157 |
| Mean annual low flow (L/s) | 28 | 283 | 430 |
| Mean annual high flow (L/s) | 283 | 4246 | 7 150 |
| Slope (m/m) | 0.1 | 0.02 | 0.01 |
| Site length (m) | 300 | 1500 | 22 000 |
| Number of channel units | 28 | 245 | 123 |
| Low flow wetted width (m) | 4.5 | 10 | 40 |

weight, milligrams), measured (total length, centimetres), and returned to the appropriate channel unit.

Fish assemblages differ greatly among the three stream sites. Mack Creek contains only cutthroat trout. Lookout Creek contains cutthroat trout, rainbow trout, chinook salmon (*Onchorhynchus tshawytscha*, rare), longnose dace (*Rhinichthys cataractae*), speckled dace (*Rhinichthys osculus*), mottled sculpin (*Cottus bairdi*), paiute sculpin (*Cottus beldingi*), and largescale sucker (*Catastomas macrocheilus*). South Fork contains cutthroat trout, rainbow trout, bull trout (*Salvelinus confluentus*), chinook salmon, mountain whitefish (*Prosopium williamsoni*), longnose dace, speckled dace, several suckers (*Catastomas* spp.), and possibly some *Cottidae* spp.

This study focuses on the distribution of adult cutthroat and rainbow trout during autumn low flow because the majority of distribution data were collected during this period. Cutthroat trout predominate in the headwater streams, mix with rainbow trout further downstream, and are gradually replaced by rainbow trout in the lower reaches. Cutthroat and rainbow trout occupy similar habitat and it has been speculated that there is some interbreeding where they co-occur. We grouped rainbow and cutthroat trout together when determining distributions in relation to physical habitat. Dace and sculpins may comprise a greater proportion of total fish biomass than trout in Lookout Creek and South Fork but they are difficult for divers to observe, and sculpins are also difficult to electroshock. For the purposes of this study, we assumed that dace and sculpins do not influence trout distributions. Although this study does not focus on seasonal changes in fish distributions, differences in fish size-class distributions, or the effects of inter- and intra-species interactions, the GA can be used for these types of analyses if the required data are available.

### Physical measurements

Survey crews collected physical data from all three stream sites during summer and autumn 1987 to develop a hierarchical template extending from the landscape to stream channel unit scale (Frissel et al. 1986; Gregory et al. 1991). Within the landscape, the drainage basin represents the broadest hierarchical division and can be subdivided into sections, reaches, and channel units. Sections are typically

**Fig. 3.** Sample reproduction and crossover strategies to produce two new offspring from two parents. The top two formulae and associated coefficient strings represent the parents. Without crossover, exact copies of the parents would be used as the two new offspring. If crossover occurs, it can take place in both the formula and coefficient string. The bottom two formulae and associated coefficient strings represent an example of how the two new offspring might be produced through crossover (i.e., recombination) of a portion of the parent coefficient strings and formula subtrees.

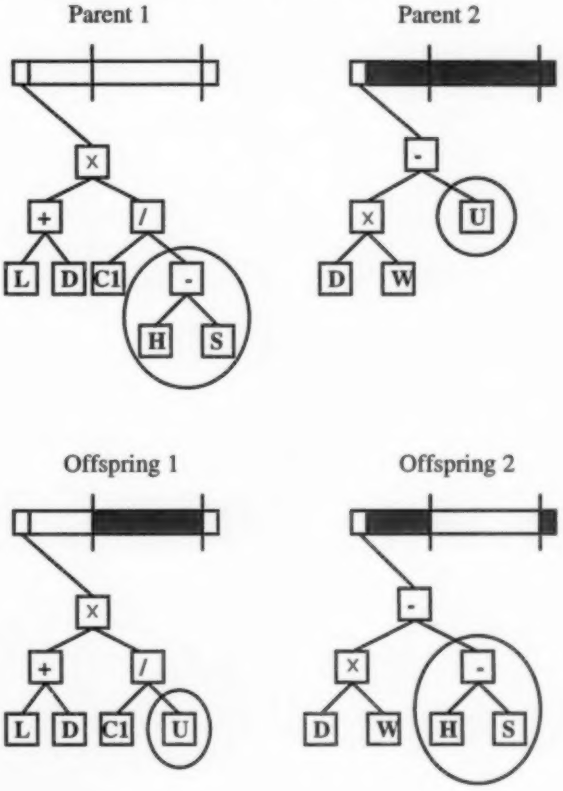
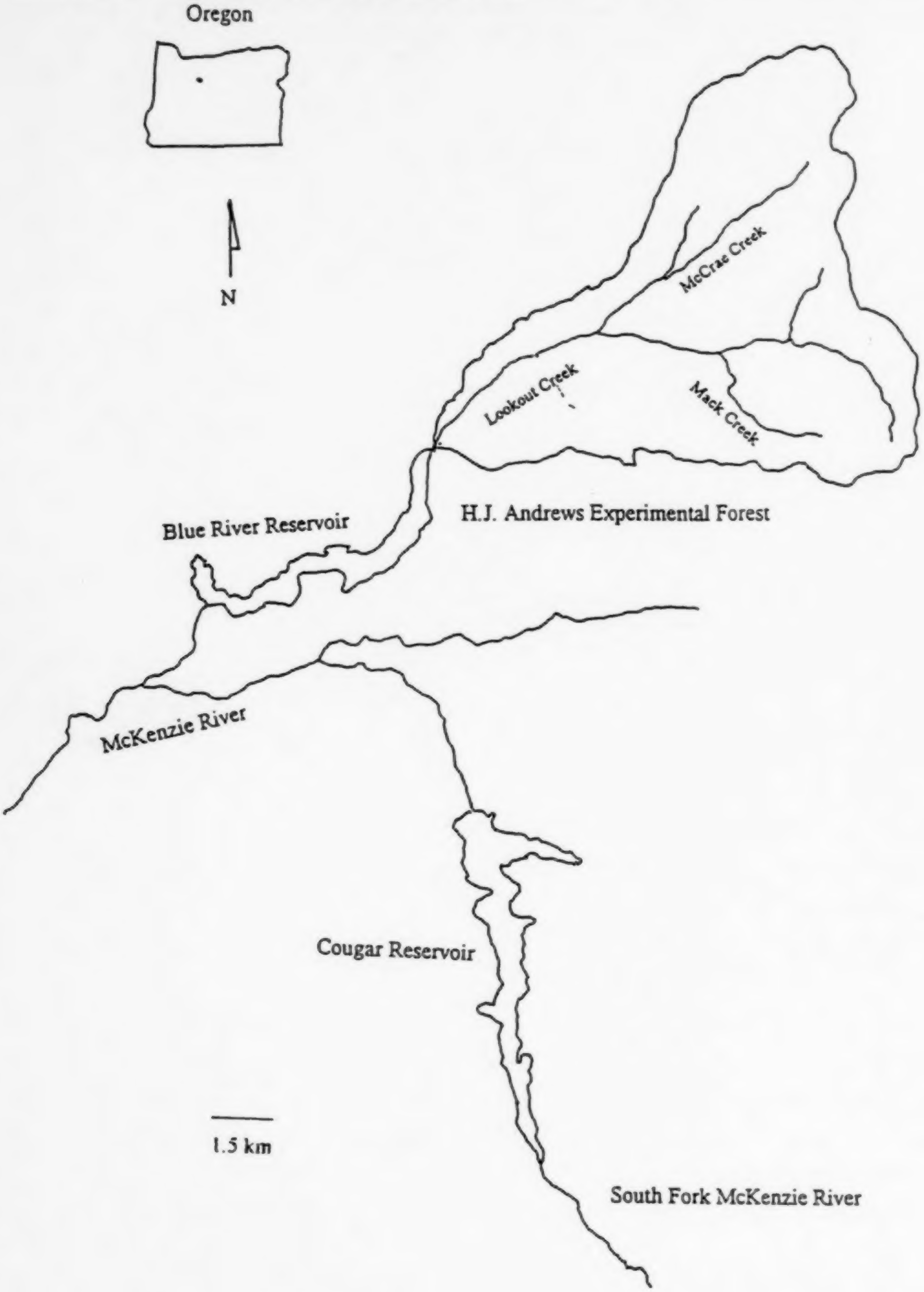


**Fig. 4.** Map showing the H.J. Andrews Experimental Forest and locations of the three streams: Mack Creek, Lookout Creek, and the South Fork McKenzie River.


Oregon
N
McCrae Creek
Lookout Creek
Mack Creek
H.J. Andrews Experimental Forest
Blue River Reservoir
McKenzie River
Cougar Reservoir
1.5 km
South Fork McKenzie River

on the order of tens of kilometres in length and are delineated by geologic and topographic discontinuities such as transitions between montaine, piedmont, and lowland areas. Within sections, reaches are defined on the basis of constraint by valley walls and are typically hundreds of metres in length. Unconstrained reaches are characterized by a valley floor width that is at least double the active channel width (i.e., the wetted width at bankfull flow) and often have a braided channel. Constrained reaches tend to be straight, single channels within narrow valleys. Reaches may also be semiconstrained reaches (i.e., constrained on one side of the stream), split channel (containing two or more approximately equal branches of the stream), or barrier (containing some impediment to upstream or downstream fish movement). Reaches are, in turn, comprised of series of channel units that are distinct erosional or depositional bedforms that can be delineated according to water-surface slope, width-to-depth ratio, water velocity, and surface turbulence (Grant et al. 1990). Channel units in order of increasing water-surface slope, water velocity, and surface turbulence are as follows: pools, glides, riffles, rapids, cascades, and steps. Pools and glides are differentiated by depth distribution (i.e., pools have an area of deeper scour whereas glides are uniform in depth). Riffles and rapids are characterized according to surface turbulence and substrate size: riffles are less turbulent and have a smaller substrate size (e.g., gravel to small cobble) than rapids (e.g., large cobble to small boulders). Cascades are steep, turbulent channel units and typically exhibit short within-unit pool–step sequences with large substrates (e.g., fields of boulders). Steps or waterfalls are very steep channel units or discontinuities characterized by turbulent water and a slope typically greater than 1 m/m.

Survey crews walked the length of each stream site and identified channel unit types. For each channel unit, crews measured or estimated (using Hankin and Reeves 1988 estimation techniques) length, wetted width, active width (i.e., predicted width at average bankfull level), mean depth, maximum depth, stream bank height to the first floodplain terrace, and floodplain width (i.e., distance to the hillslope). Wetted and active channel width were calculated as an average of upstream, middle, and downstream channel unit widths. Average depth was calculated from measurements taken at approximately 10 locations along three transects perpendicular to the flow or from five or six random measurements. Percent riparian cover, stream bank height to terrace, and terrace width were estimated visually. Degree of channel constraint was determined from the ratio of terrace width to active channel width. Reach slope was measured with a hand-held clinometer. Number and dimension (length, diameter) of wood pieces were also noted, and dominant substrate was classified with a modified Wentworth scale (bedrock, large boulder, small boulder, large cobble, small cobble, gravel, sand, and silt).

### Data analyses

We used three different procedures (proportional, MLR, and GA) to examine relationships between adult trout distributions and stream physical characteristics. All analyses of Lookout Creek were based on fish number because no fish biomass data were available. Biomass for South Fork was calculated from length-to-weight regressions for other streams in the basin. Note also that because fish lengths for South Fork were estimated by divers, biomass values are estimates. Both numbers and biomass data were available for Mack Creek.

For procedure 1, trout were distributed proportional to the surface area or volume of each channel unit to determine if trout distributions were determined solely by territory size. Previous studies have shown that adult trout exhibit territorial behavior, with the dominant trout commonly occupying a position near the upstream end of a channel unit and subdominant fish forming a triangular arrangement behind the dominant fish (Jenkins 1969). Proportional distributions (fish per square metre and per cubic metre) were calculated by taking the total number of fish in each stream and dividing by the total surface area or volume. The number of fish predicted per channel unit was then determined by multiplying the number of fish per square metre or per cubic metre by the surface area or volume of each channel unit. Correlations between observed and predicted distributions were calculated.

Procedure 2 used MLR analyses to relate observed trout distributions to the physical characteristics of each stream (e.g., reach type, unit type, substrate, channel unit dimensions). Because there was little difference between the multiple correlation coefficients ($R$ values) or equations of the three MLR methods (MLR, forward and backward stepwise and $r^2$ methods; SAS Institute Inc. 1985) and review of the literature revealed that the backward stepwise method without interaction terms is most commonly used, we report results from the backward stepwise method here. MLR statistics are reported as $R$ values rather than $R^2$ values to facilitate comparison with GA correlations.

For procedure 3, we used the GA to evaluate the data and develop an equation relating trout distributions to stream reach and channel unit characteristics. The specific design of our GA is as follows. GAs were developed by creating a population of 200 individual formulae to distribute fish among channel units. Each of the 200 individuals in the GA population was a formula comprised of up to 25 independent variables (e.g., channel unit type, unit length, unit depth, pieces of wood, and substrate type) and mathematical operators (i.e., plus, minus, product, quotient, $x^y$, square root) with coefficients for non-numeric variables (e.g., channel unit type). GA formulae could also include as variables the channel unit type and associated characteristics of adjacent upstream and downstream channel units to determine if the number of fish in a unit is influenced by adjacent units. The GA replicated and recombined formulae and coefficients and evaluated the fitness of each equation. We designed fitness to maximize $R$ value and minimize the sum of squares for correlations between number of trout predicted and number of trout observed in each channel unit. For each generation, those formulae with a high fitness value had a greater likelihood of being the two parents chosen for reproduction. The GA was run until formula improvements reached a plateau (about 20 000 generations) and the best individual in the population was then selected as the final equation for that run. This procedure was then repeated 10 times for each stream and the best-fitting formula (i.e., the formula with the best fitness

**Table 2.** Correlation values ($r$) for trout observed in each channel unit compared with trout predicted to be in each channel unit, using surface area and volume distributions, multiple linear regression equations (MLR), and genetic algorithm (GA) distributions.

| | Mack Creek | | | South Fork | |
|---|---|---|---|---|---|
| Procedure | Number | Biomass | Lookout Creek (number) | Number | Biomass |
| Surface area | 0.82 | 0.74 | 0.53 | 0.33 | 0.44 |
| Volume | 0.81 | 0.81 | 0.52 | 0.26 | 0.58 |
| MLR | 0.91 | 0.89 | 0.65 | 0.61 | 0.61 |
| GA mean | 0.95 | 0.94 | 0.72 | 0.73 | 0.68 |
| GA standard error | 0.01 | 0.02 | 0.04 | 0.03 | 0.04 |

**Note**: The $r$ values are presented for MLRs to facilitate comparisons with proportional and GA correlations.

**Table 3.** Habitat type variables and equations used in the multiple linear regressions to predict trout distributions (number/channel unit) at each stream site.

| | Variable | Equation |
|---|---|---|
| Mack Creek | Unit type<br>Unit length<br>Percent cover | Mack Creek trout/channel unit = −3.38 + 3.97(length) − 0.35(percent cover) + 29.69(unit type pool) + 27.68(unit type riffle) + 29.98(unit type rapid) |
| Lookout Creek | Unit length<br>Unit width<br>Unit depth<br>Pieces of wood | Lookout Creek trout/channel unit = −3.4 + 0.19(length) − 0.14(active channel width) + 0.33(pieces of wood) + 0.51(unit width) + 12.14(unit depth) + 32.97(unconstrained reach type) |
| South Fork | Unit length<br>Active channel width<br>Reach type | South Fork trout/channel unit = 1.57 + 0.09(length) − 0.28(active channel width) + 13.87(unconstrained reach type) |

value) from the 10 runs was selected as the final solution equation and used to estimate trout for each stream unit. The difference between fitness values was typically less than 10%. If two equations had the same fitness value, we selected the shorter of the two equations. A compiled version of the GA is available on diskette from the primary author.

We also wanted to evaluate the predictive abilities of the proportional, MLR, and GA equations on similar data sets and the transferability of GA equations between diverse data sets. The predictive abilities of proportional methods, MLRs, and GAs on similar data sets were tested on Lookout Creek data by splitting the data into two parts. Using these methods we developed GA equations for part of the data (training sets) and then applied the GA equations to predict trout distributions in the second part of the data (testing sets). Partial data sets were created by removing from the main data set two out of three (2:1), every other (1:1 ratio of training to testing data), every third (1:2 ratio), every fourth (1:3 ratio), or every fifth (1:4 ratio) representative of each channel unit type and using the removed data to create a training set. To determine the transferability of GA equations between diverse data sets, we used the equation developed for one stream to distribute fish in the other two streams and compared observed with predicted distributions. We also combined variables from all three equations into a new general equation, provided the GA with the new equation, and let it determine coefficients for each stream site.

## Results

### Proportional distributions

Correlations between observed trout (number and biomass per channel unit) and trout predicted from proportional distributions were significant for all streams ($P < 0.05$), but $r$ values were low (i.e., <0.6) in Lookout Creek (5th order) and South Fork (7th order) (Fig. 5). Correlations between number of trout observed and trout distributed based on surface area produced similar or slightly higher $r$ values than trout distributions based on volume, whereas biomass was predicted more effectively by volume (Table 2).

### Multiple linear regressions

MLRs were run on the Mack Creek, Lookout Creek, and South Fork data sets (Fig. 6). As with surface area and volume distributions, MLRs produced the best relationships for Mack Creek and the worst for South Fork

**Fig. 5.** Trout predicted to be in each channel unit on the basis of channel unit surface area compared with trout observed in each channel unit for Mack Creek, Lookout Creek, and South Fork McKenzie River.

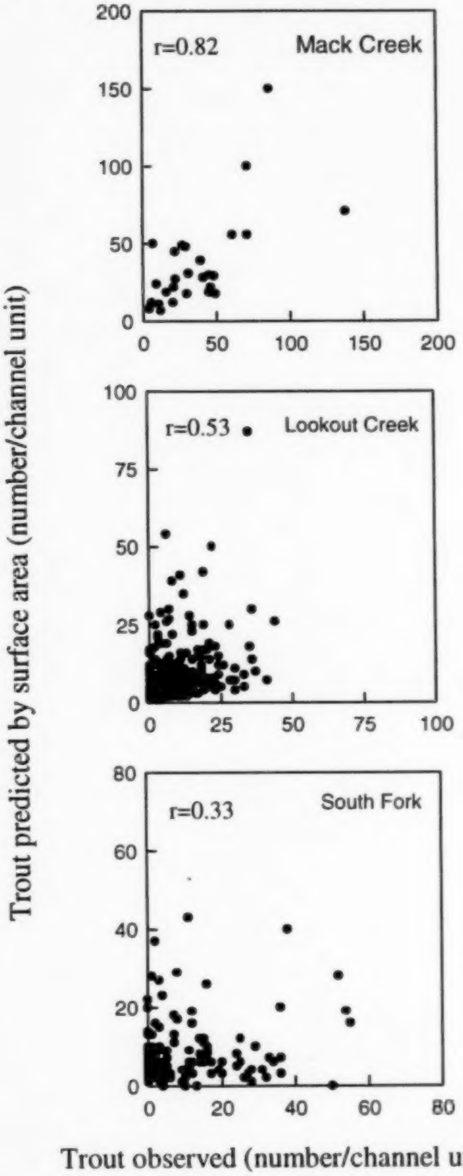
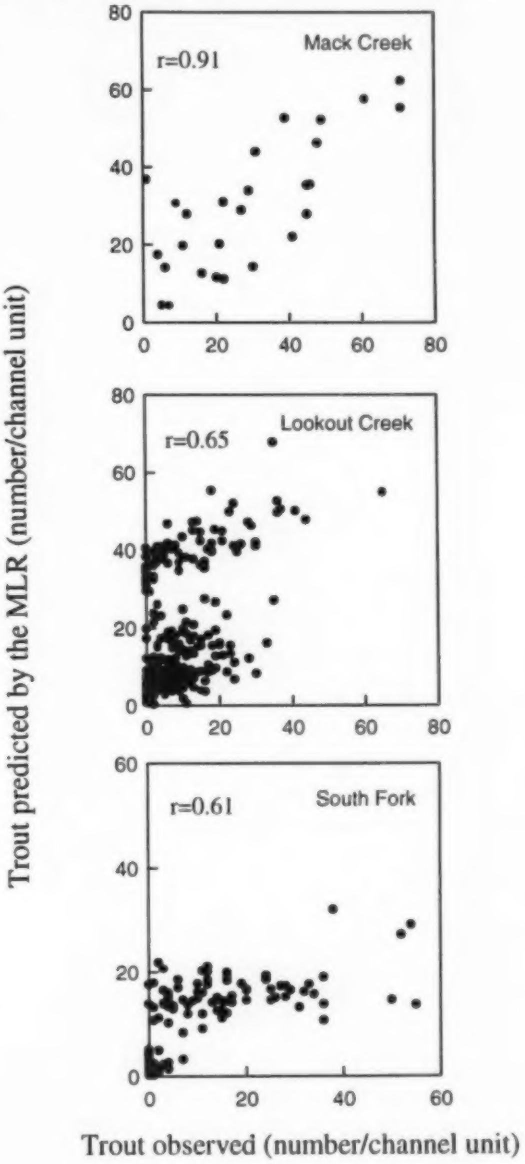


**Fig. 6.** Trout predicted to be in each channel unit on the basis of MLR equations compared with trout observed in each channel unit for Mack Creek, Lookout Creek, and South Fork McKenzie River.



(Table 2). MLR habitat variables and equations used to produce these distributions are listed in Table 3.

### Genetic algorithms

The GA was also run on each stream and provided significantly better distribution equations than the other methods (paired *t* test, $P < 0.05$) (Fig. 7, Table 2).

### Predictive abilities

We evaluated the ability of surface area, the MLR, and the GA to develop a distribution equation from one portion of a data set and predict the distribution of trout in another portion of a data set by separating the Lookout Creek data into training and testing sets of various sizes. We used the Lookout Creek data because it contains more complexity

**Fig. 7.** Sample correlation between trout predicted to be in each channel unit by the stream-specific genetic algorithm equation (GA) compared with trout observed in each channel unit for Mack Creek, Lookout Creek, and South Fork McKenzie River.

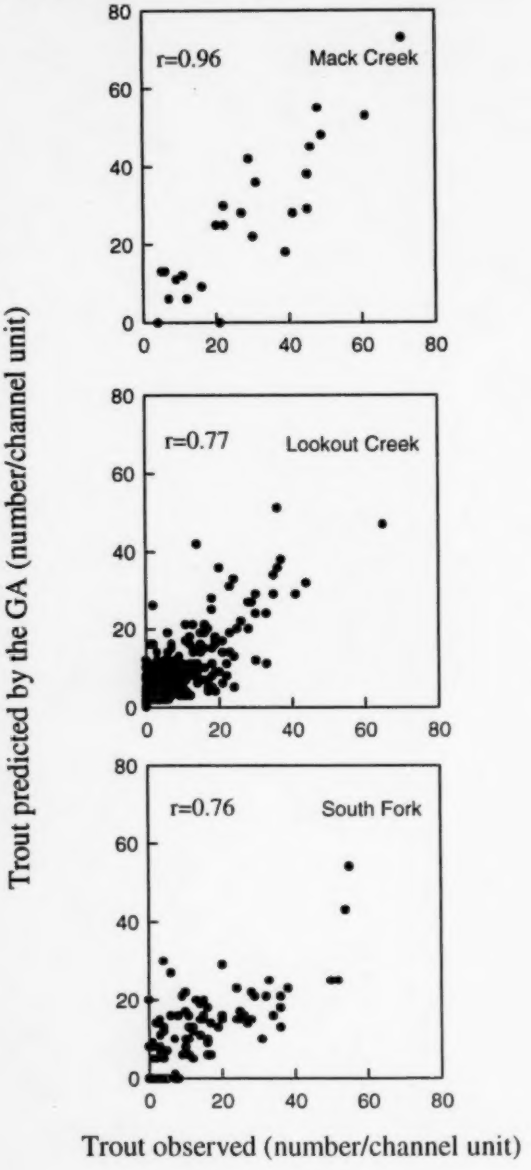
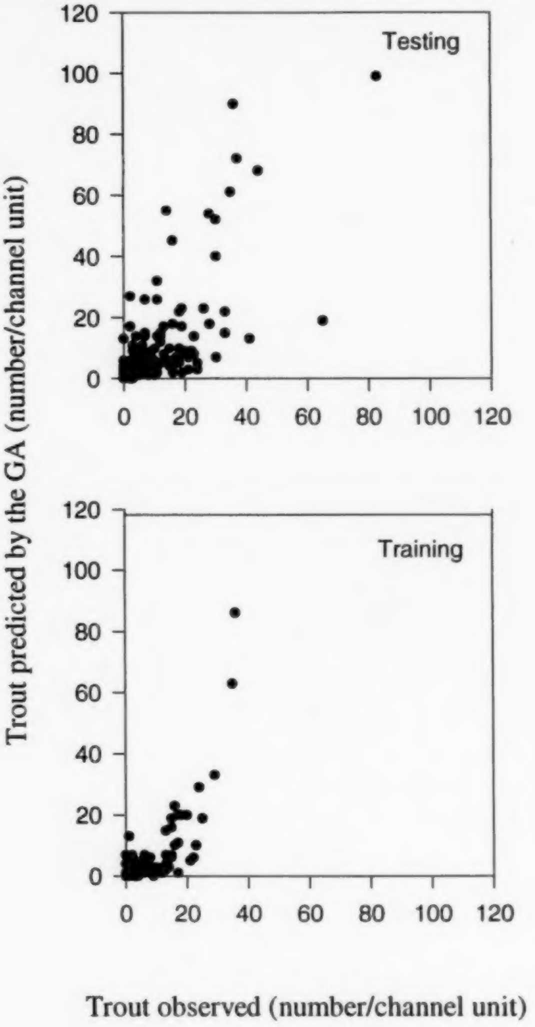


**Fig 8.** Sample correlation between trout predicted to be in each channel unit by the genetic algorithm (GA) equation compared with trout observed in each channel unit for Lookout Creek training and testing sets with a 2:1 ratio (i.e., two out of every three of each unit type were selected for the training set and the remaining one out of three units was used in the testing set).



than Mack Creek, but without the complications of the diverse fish community and flood-control dam in South Fork. We used training and testing sets of different sizes to determine how the ratio of training to testing set size influenced performance. For each method, we used the training data to develop an equation relating trout number to stream physical characteristics. We then used this equation to relate trout distributions to stream physical characteristics for the remainder of the data (i.e., the testing portion). For example, if one out of three units was used to develop an equation (i.e., for training), then the remaining two out of three units were used to test the equation.

Fish per square metre calculated proportional to surface area from the training set data did not effectively predict the number of fish per square metre (i.e., *r* values were below 0.4) in the testing sets. MLRs for the training sets were effective when the ratio of the training set to the testing set was 2:1 but less effective for other ratios

**Table 4.** Correlation values ($r$) for trout observed in each channel unit compared with fish predicted to be in each channel unit in proportion to surface area, a multiple linear regression (MLR), and a genetic algorithm (GA) equation developed for part of the Lookout Creek data (training set) and used to predict distributions in the other part of the Lookout Creek data (testing set).

| | Surface area | | MLR | | GA | |
|---|---|---|---|---|---|---|
| Training:testing ratio | Training | Testing | Training | Testing | Training | Testing |
| 1:4 | 0.02 | 0.04 | 0.65 | 0.45 | 0.76 | 0.38 |
| 1:2 | 0.45 | 0.40 | 0.77 | 0.59 | 0.79 | 0.55 |
| 1:1 | 0.29 | 0.24 | 0.63 | 0.51 | 0.78 | 0.54 |
| 2:1 | 0.24 | 0.20 | 0.61 | 0.66 | 0.71 | 0.72 |
| 4:1 | 0.33 | 0.29 | 0.64 | 0.50 | 0.70 | 0.58 |

**Note**: The Lookout Creek data were split into testing and training portions of various sizes, where 4:1 indicates that four out of five of each unit type were selected for the training set and the remaining one out of five units were used in the testing set. Similarly, 1:4 indicates that one out of five of each unit type was selected for the training set, and the remaining four out of five units were used in the testing set. $R$ values are presented for MLRs to facilitate comparisons with proportional and GA correlations.

(Table 4). The GA was also most effective with a training to testing size ratio of 2:1 (Table 4, Fig. 8) and was a somewhat better predictor than the MLR in three out of the five data sets. Also note that the $r$ value for the testing set may be higher than the $r$ value for the training set if the equation produced by the training set happens to be more effective at predicting testing set data (e.g., data sets with a ratio of 1:4 using surface area equations and 2:1 using GA equations; Table 4).

To further test the GA, we also wanted to determine if a GA equation and coefficient set from one stream could be applied in other streams that contained similar or different sets of physical characteristics. Therefore, we used the stream-specific GA equations developed for each stream to predict trout distributions in the other two streams. Habitat variables used by the GA, the general GA equation, and coefficients for the Mack Creek example are in Table 5. The equation for Mack Creek was not effective at predicting trout in Lookout Creek or South Fork (i.e., produced $r$ values less than 0.4; Table 6). The equation for Lookout Creek was moderately effective at predicting the distribution of trout in Mack Creek and less effective for South Fork (Table 6). The South Fork equation was ineffective in both Mack and Lookout creeks (Table 6).

Additionally, we wanted to determine if a general equation could be developed that would predict the distribution of fish in all three streams. To attempt this we combined variables from the three stream-specific equations into a new general equation, provided the GA with the new equation, and let it determine coefficients for each stream site. Stream-specific coefficients allowed the GA to include or exclude portions of the formula. The GA was then used to distribute trout in all three stream sites using a single equation and a set of coefficients specific to each stream site. Using the more generic equation with stream-specific coefficients, the GA performed much better than it did using a single equation and coefficient set for all three streams and as well as it did with three separate equations (Table 6, Fig. 9).

## Discussion

Our study revealed that GAs are useful for discovering relationships in complex data. GA predictions were typically more effective than proportional and MLR predictions. Each method, however, evaluated the data from a slightly different perspective and thereby provided different insights into trout–habitat relationships.

Distributions based on surface area and volume were effective for Mack Creek, which is physically complex at the channel-unit scale but spatially uniform at the scale of reach and above. The section of Mack Creek that we studied was constrained, had a relatively uniform width (4 ± 1.5 m; mean ± SD), and contained primarily pool or cascade sequences with a boulder substrate (even in pools). Distribution of wood was patchy, but crevices between boulders provided an additional source of cover, so fish were not measurably dependent upon wood availability. The prevalence of good, uniformly available habitat allowed fish to distribute largely on the basis of surface area or volume.

Distributions in Lookout Creek and South Fork were less predictable on the basis of surface area or volume because the study sections of Lookout Creek and the South Fork contain diverse reach types with single and braided channels. Lookout Creek also contains the widest variety of channel unit types, with representatives of all six types (pools, glides, riffles, rapids, cascades, and steps). South Fork is dominated by pools and riffles, but is complicated by a diverse geology and a flood-control dam. Additionally, Mack Creek contains only cutthroat trout, whereas Lookout Creek and South Fork have a much greater diversity (including anadromous steelhead trout and chinook salmon in the South Fork). For all streams, surface area was a better predictor of fish number than volume because many units (including pools in Mack Creek) were less than 0.4 m deep, thereby precluding vertical habitat partitioning. In South Fork, which did have deeper units, volume was a more effective predictor of fish biomass, perhaps because

**Table 5.** Habitat variables used by the genetic algorithm to predict trout distributions at each stream site along with the coefficient for each stream site.

| | Mack Creek | Lookout Creek | South Fork |
|---|---|---|---|
| Variables | Unit type, up, down<br>Unit length<br>Unit depth | Unit type, up, down<br>Unit length<br>Unit depth<br>Pieces of wood<br>Substrate type, up, down<br>Reach type | Unit length<br>Unit depth<br>Pieces of wood<br>Substrate type, up, down<br>Reach type |
| General coefficients | | | |
| Coefficient 1 | −0.61 | −2.41 | −5.27 |
| Coefficient 2 | 3428.74 | 1.15 | 2.24 |
| Coefficient 3 | 1.0 | 0.94 | 1.05 |
| Unit types | | | |
| Pools | 0.32 | 1.72 | −773.51 |
| Up-unit | 0.83 | 4.07 | −401.74 |
| Down-unit | 16.8 | 3.74 | 959.51 |
| Riffles | 28.32 | 6.22 | −128.81 |
| Up-unit | 4.08 | 7.21 | −498.22 |
| Down-unit | −3.63 | −7.79 | 226.8 |
| Rapids | 12.42 | 15.01 | −789.8 |
| Up-unit | 17.88 | 11.66 | −491.2 |
| Down-unit | 10.5 | 22.17 | 177.1 |
| Cascades | −3.39 | 1.99 | 0.06 |
| Up-unit | 19.02 | −3.38 | 724.65 |
| Down-unit | 7.55 | 1.56 | −367.48 |
| Steps | 10 | −48.2 | −173.62 |
| Up-unit | 15.89 | 16.26 | −253.27 |
| Down-unit | 21.0 | 18.13 | −2782.89 |
| Reach types | | | |
| Constrained | 1.23 | 0.70 | 0.62 |
| Unconstrained | 0 | 2.38 | 663.7 |
| Semiconstrained | 0 | 1.16 | 1.10 |
| Split channel | 0 | 0.46 | 0.57 |
| Barrier | 0 | 1.17 | −0.03 |
| Substrate types | | | |
| Cobble–gravel | 0 | 0 | 321.5 |
| Up | 0 | 0 | 101.57 |
| Down | 0 | 0 | 10.86 |
| Cobble | 0 | 0 | 27.90 |
| Up | 0 | 0 | 55.90 |
| Down | 0 | 0 | 51.34 |
| Small boulder | 3.58 | −11.65 | 9.20 |
| Up | −125.5 | 10.45 | 19.74 |
| Down | 3.58 | 10.83 | −6.45 |
| Bedrock | 0 | 0 | 273.13 |
| Up | 0 | 0 | −25.71 |
| Down | 0 | 0 | 0 |

**Note**: The general equation is trout-channel unit = (reach type × (unit type up + unit type + unit type down) × (pieces of wood + coefficient 2) × $\sqrt{(\text{length} \times \text{depth} + \text{coefficient 1})}$ × (substrate up + substrate + substrate down)) + coefficient 3. "Up" or "down" indicates that this variable for the adjacent upstream or downstream unit, respectively, is also included in the equation. Each stream has three general coefficients and a coefficient for each non-numeric term in the equation. Extremely large coefficients that are added to small numerical variables eliminate the effects of that term in the equation. When all coefficients but one are zero, that term is also eliminated from the equation because there is only one type present in the stream (i.e., only constrained reaches are present in the section of Mack Creek that was surveyed). Positive and negative coefficients must be evaluated in the context of the equation to determine their impact.

**Table 6.** Correlation values (*r*) for number of trout observed in each channel unit compared with the number of trout predicted to be in each channel unit using a genetic algorithm equation developed for one stream (training stream) to predict trout distributions in the other two streams (testing streams) and a single general equation with stream-specific coefficients (see Table 5) used to predict distributions in all three streams.

| | Stream | *r* | Stream | *r* | Stream | *r* |
|---|---|---|---|---|---|---|
| Training data | Mack | 0.95±0.01 | Lookout | 0.72±0.04 | South Fork | 0.73±0.03 |
| Testing data 1 | Lookout | 0.32±0.03 | Mack | 0.53±0.21 | Mack | 0.24±0.19 |
| Testing data 2 | South Fork | 0.15±0.09 | South Fork | 0.38±0.14 | Lookout | 0.18±0.15 |
| General equation | Mack | 0.94 | Lookout | 0.75 | South Fork | 0.75 |

**Note:** Values are calculated for 10 runs and are given as the mean ± SE.

larger fish require and (or) prefer deeper water. Channel unit surface area and volume may also be relatively ineffective predictors for Lookout Creek and South Fork because channel unit size increases from Mack Creek to South Fork. In Lookout Creek and South Fork, pools were larger and fish were not using the center portion of the unit. We observed fish primarily at the heads and tails of large pools. The few fish observed in the midsections were often moving between the head and tail sections.

Both MLRs and GAs provided better results than proportional distributions. MLRs, however, are hindered by criteria of independence of variables and model linearity that are unlikely to be met. Assuming that violations of linearity and independence did not influence conclusions drawn from the statistics (a large assumption in many cases), MLRs identified important physical determinants of fish distribution and explained 80% of the variance for Mack Creek, 42% for Lookout Creek, and 37% for South Fork. The GA was more effective, explaining 94% of the variance for Mack Creek, 68% for Lookout Creek, and 56% for South Fork, without assumptions of linearity or independence of variables.

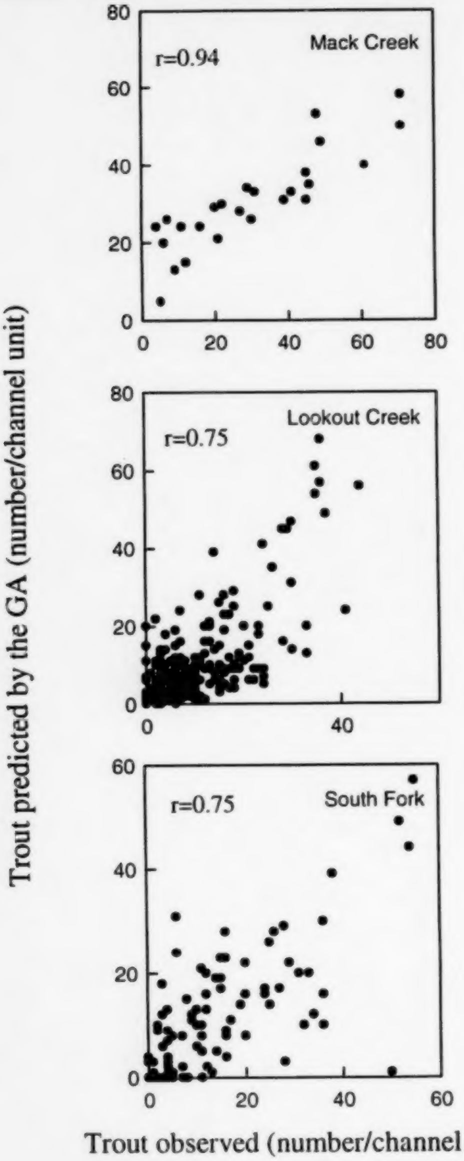
The MLR and GA included similar, but not identical, variables in the distribution equations (Tables 3 and 5). For Mack Creek, which is uniformly constrained and boulder dominated, the MLR (stepwise) included unit type, length, and percent cover in the regression equation. The GA weighted coefficients to include unit type, length, and depth. Inclusion of unit length in all equations supports the conclusion that channel unit surface area is a relatively good predictor of the fish distribution in this stream. Width was not included in the equations because it is relatively constant in Mack Creek. The addition of channel unit type and percent riparian cover by the MLR, and unit type and depth by the GA improved predictions compared with those based only on surface area. Wood was not included in the Mack Creek equations, presumably because crevices in the large boulder substrate provide an alternative source of cover.

For Lookout Creek, which has diverse reach types and a smaller substrate, the MLR included channel unit length, width, depth, number of pieces of wood, and reach type. The GA coefficients included the same variables as the MLR but added unit type and excluded unit width from the equation. Wood is more important in Lookout Creek than in Mack Creek because many units have smaller substrates and wood is therefore required for cover. Both the MLR and GA noted that unconstrained reaches contained relatively more fish than other reach types. The greater abundance of fish in unconstrained reaches may be related to a greater diversity of flow regimes, microhabitats, or a larger food supply (Moore and Gregory 1989; Gregory et al. 1991).

South Fork has diverse reach and substrate types but lacks a diversity of channel unit types. For South Fork the MLR included unit length, active channel width, and reach type. Note that while fish distributions based solely on surface area were poor for South Fork ($r = 0.33$) the inclusion of reach type by the MLR equation increased the $R$ value to 0.61. The GA weighted channel unit length, depth, wood, substrate type, and reach type as important and eliminated unit type to obtain an average $r$ value of 0.75.

In addition to evaluating the equations for each stream, we wanted to determine if the same factors that determine trout distributions in headwater streams also determine distributions in downstream reaches, or conversely, if the factors that determine trout distributions in headwater streams are different from those in downstream reaches. MLR equations hinted that a longitudinal transition of variables might exist, but did not consistently include or exclude variables so that longitudinal changes in trout–habitat relationships were obvious. We used the GA to further examine the possibility that the importance of habitat variables might change from a 3rd-order to a 7th-order stream in a predictable manner. The GA was used to develop a general equation that included all of the variables identified by the best stream-specific equations and that could be adjusted with stream-specific coefficients. Coefficients for the general equation go to zero or a very large number to eliminate the effects of a variable. Evaluation of the GA formula and coefficients revealed a more apparent longitudinal shift in importance from unit type and length at the upstream site (Mack Creek), to an inclusion of reach type at the intermediate stream site (Lookout Creek), and finally a loss of importance of unit type at the downstream site (South Fork; Table 5). In the streams of the McKenzie River basin dominated by basalt boulder and cobble, small scale geomorphic features are important in small streams, while a combination of small (unit type) and larger scale geomorphic features (reach type)

**Fig. 9.** Trout predicted to be in each channel unit by the general genetic algorithm (GA) equation compared with trout observed in each channel unit for Mack Creek, Lookout Creek, and South Fork McKenzie River.



and physical properties (wood) are important in intermediate sized streams. Large-scale geomorphic features and basic physical properties (substrate and wood) dominate in larger streams. This pattern is not as apparent from the MLRs because there was no clear transition of variables from the upstream site to the downstream site in the MLR equations (Table 3).

The ability of the GA to include interactions between adjacent upstream and downstream channel unit characteristics may have contributed to its success in these applications. While the effects of upstream and downstream channel units can be included in the MLR, thereby tripling the size of the data set, when we included this large number of variables it seemed to "confuse" the MLR. Equations produced by the MLR when upstream (up) and downstream (down) variables were included often had little biological meaning (e.g., fish per square metre = 300 + 20.0(length) − 11(length down) − 70(active channel width up) + 53.3(active channel width down) − 162(channel unit riffles down) + 20.5(wood down). While it seems quite logical that fish are influenced by upstream and downstream channel unit characteristics, it is unlikely that their distribution is governed almost entirely by upstream and downstream channel unit characteristics as the large coefficients for active channel width upstream, active channel width downstream, and channel unit riffles in this MLR equation would suggest. Furthermore, *R* values from equations produced by the MLR when upstream and downstream variables were included were not significantly better (paired $t$ test, $P < 0.05$) than MLR equations without upstream and downstream variables.

GAs, which were specifically designed to handle large numbers of variables (i.e., large solution search spaces), were capable of revealing the potential importance of upstream and downstream characteristics. Performance of the GA achieved by inclusion of interactions between adjacent channel units demonstrates the need to emphasize exploration of how interactions between environmental variables affect biotic responses. Further examination of how channel unit sequences influence the distribution of trout has the potential to greatly enhance our understanding of how trout respond to their environment.

To evaluate the ability of equations developed with the GA, MLR, and proportional distributions to "learn" from one data set and predict distributions in a similar data set, we split the Lookout Creek data into training and testing portions. Evaluations of testing and training set data revealed that GAs explained training data significantly better than the MLR (paired $t$ test, $P < 0.05$) but did not predict testing data significantly better. For the Lookout Creek partial data sets, the MLR explained 38% of the variability in the training sets and about 25% of the variability in the testing sets. On average, the GA was more effective, predicting 56% of the variability in the training sets and 31% of the variability in the testing sets. Note that the training set contained few units with more than 40 fish (Fig. 8). Consequently, the GA was not effective at predicting the two units in the testing set that had the greatest number of fish. This analysis indicates that while GAs have potential as a predictive tool and may ultimately be useful for reducing field sampling efforts, they must be carefully developed with data from comparable streams and not extrapolated beyond the values used in their development.

We further evaluated the GA's predictive capabilities by attempting to use a GA formula and equation set from one stream to predict fish distribution in the other two streams. This exercise allowed us to determine under what circumstances GA distribution equations are transferable.

Mack Creek and South Fork, which each contained only a subset of all geomorphic and physical properties, produced poorly transferable equations. Lookout Creek contained a more representative sample of all geomorphic and physical properties and was therefore more capable of producing an equation that could be extrapolated, with some effectiveness, to the other streams. The more disparity there is between habitat variables (e.g., wood, unit types, substrate types) and frequency of occurrence, the less effective the GA was at developing a transferable equation. The use of a general equation with coefficients specific to each stream improved the model's fit to the data and supports the contention that all important components of the system must be represented by the analyses. Accordingly, equations should be developed from data that contain a representative sample of important components and only be extrapolated to data that contain similar components.

The GA in this study identifies important habitat areas for trout in the McKenzie River basin and may help resource managers weigh potential impacts of habitat alteration strategies. For this application, the GA had greater flexibility than a MLR in its choice of equations and was more capable of handling a larger numbers of variables. As a result, the GA more effectively explained fish distributions. Our GA was designed and implemented using data available from previous surveys. Fisheries managers could use these variables or a wide variety of others (e.g., water velocity in each channel unit, size distributions of fish in each unit, and habitat provided by undercut banks or shading from riparian cover) to characterize their stream system. The GA could also be altered to predict relationships such as total biomass supportable by streams of a given type, or the influence of species interactions on fish distributions. For example, a GA could be developed to evaluate relationships between stream physical characteristics and total fish biomass for numerous streams in a region to determine if certain stream characteristics (e.g., summer and winter habitat availability, spawning gravel) are associated with large fish standing stocks, or perhaps more importantly, with very low fish standing stocks.

GAs have wide applicability to the study of ecological relationships, both as exploratory and explanatory tools. GAs are flexible enough to accommodate a wide variety of study designs without the statistical constraints of independence and linearity; thus, they are a useful technique for relating the distributions of organisms to their physical and biological environment. A priori understanding of system relationships is not required. For fisheries researchers and managers, GAs may provide a new way to develop expert systems. Traditional expert systems must be specifically designed for each management issue, category of stream types, and group of species interactions. With a GA, a new expert system could be designed by providing an existing GA with the input and output data sets for which a relationship is desired. Using the input data provided, the GA learns which relationships can produce the desired outcomes. The programmer does not impose static constraints on model design and operation, but rather defines the direction of function evolution by determining the fitness optimization criteria (i.e., $r$ values and sum of squares for this study). The fisheries manager could then evaluate the GA equations or sets of rules to assure its biological relevance, and readjust the GA as necessary. For more research-oriented exploration, the dynamic characteristics of GAs also make them an effective tool for generating hypotheses from descriptive surveys that may be successful avenues for experimental analysis.

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# Effect of age, growth rate, and ontogeny on the otolith size – fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages[1]

**Jonathan A. Hare and Robert K. Cowen**

**Abstract**: The otolith size – fish size relationship was examined in field-collected larval and pelagic juvenile bluefish, *Pomatomus saltatrix*. The purpose was to evaluate the central assumption of proportional back-calculation techniques, namely that otolith and somatic growth are in constant proportion. Age-independent variability was found between otolith size and fish size that differed between ontogenetic stages. Stage-specific growth rate effects were also identified. Finally, the otolith size – fish size relationship changed at certain ontogenetic stage transitions. These effects, as well as others that have been found, are discussed with regard to the assumption of constant proportionality between otolith growth and fish growth. In light of this discussion, the overall validity of constant proportionality becomes suspect when applied to the early life history stages of fishes. Future work should take a longitudinal approach to the analysis of the relationship between otolith growth and somatic growth. In addition, regression and proportional methods should be modified to account for growth rate and ontogenetic effects. Finally, a relative size approach is presented that is appropriate in situations that require only relative measures of fish size. This relative size approach has several benefits and these are discussed in relation to other back-calculation procedures.

**Résumé** : Nous avons examiné la relation entre la taille de l'otolithe et la taille du poisson dans des échantillons prélevés sur le terrain de larves et de juvéniles pélagiques de tassergal (*Pomatomus saltatrix*). Notre objectif était d'évaluer l'hypothèse centrale des techniques de rétrocalcul proportionnel, selon laquelle la croissance de l'otolithe et la croissance somatique seraient en proportion constante. Nous avons découvert une variabilité indépendante de l'âge entre la taille de l'otolithe et la taille du poisson, variabilité qui différait selon le stade ontogénique. Nous avons aussi repéré des effets spécifiques au stade sur le taux de croissance. Enfin, la relation taille de l'otolithe – taille du poisson changeait lors de certaines transitions entre les stades ontogéniques. Ces effets, ainsi que d'autres qui ont été observés, sont analysés par rapport à l'hypothèse de proportionalité constante entre la croissance de l'otolithe et celle du poisson. À la lumière de cette analyse, la validité globale de la proportionalité constante suscite des doutes quant à son application aux premiers stades biologiques des poissons. Les travaux futurs devraient adopter une approche longitudinale de l'analyse de la relation entre la croissance de l'otolithe et la croissance somatique. De plus, il faudrait modifier la méthode de régression et la méthode proportionnelle pour tenir compte des effets du taux de croissance et du stade ontogénique. Enfin, nous présentons une approche de la taille relative qui convient aux situations ne nécessitant que des mesures relatives de la taille du poisson. Cette approche présente plusieurs avantages, que nous examinons par rapport aux autres méthodes de rétrocalcul.

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## Introduction

The study of growth in larval and juvenile fishes has benefited greatly from the finding that increments are formed daily in the otoliths of many species. Daily increments allow the age of an individual to be determined (Brothers et al. 1976; Campana and Neilson 1985; Jones 1986). The incremental nature of otolith growth also provides a record of otolith size-at-age, from which fish size-at-age can be estimated using back-calculation techniques (see reviews by Francis 1990; Campana and Jones 1992). Such estimates of fish size can then be used to examine the role of size and growth in regulating larval fish survival, a topic of longstanding interest (see reviews by Rothschild 1986; Anderson 1988; Leggett and DeBlois 1994).

Back-calculation techniques were initially developed for the estimation of adult fish size from scale annuli, but these techniques have been used to estimate fish size from marks on a variety of other hard parts. Scale proportional techniques assume that if a scale was 10% larger at capture than the average scale of a fish of comparable size, then the individual's scale would be 10% larger than average throughout its life (the body proportional hypothesis is similar but is in terms of body size rather than scale size; see Francis 1990). It is imperative to evaluate this basic back-calculation assumption before the method is applied. Many studies examining otoliths of larval and juvenile fish have concluded that otolith growth and somatic growth are proportional on the basis of significant correlations between measures of otolith and somatic size (the term coupling has been used, but see below). These correlations indicate a general correspondence between otolith size and somatic size, but this would be expected, since the size of any two body parts will be correlated over time simply because they are both growing (Cock 1966; Shea 1985). To demonstrate a linkage between otolith size and fish size, the age-independent variability in the relationship must be assessed. Additionally, many studies have provided evidence counter to the basic assumption that otolith and somatic growth occur in a constant proportion (the term decoupling has been used, but see below). Given the potential utility of the otolith as an individual record of daily size and growth, it is important to examine the factors that might affect the otolith growth – somatic growth relationship with regard to the proportionality assumption of back-calculation. Once this is completed, the various back-calculation techniques available can be evaluated with regard to these findings.

As part of a larger program studying the processes that drive larval survival and subsequent recruitment of bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae), along the eastern coast of the United States, we are testing the hypothesis that size and growth rate are important components of survival. Accomplishing this specific test is dependent on our ability to compare the sizes of larvae at specific points in their individual histories. Before these size histories can be constructed, the otolith size – fish size relationship must be examined and the various techniques of back-calculation evaluated. The specific goals of this study were threefold. First, the age-independent variability in the otolith size – fish size relationship was examined. Second, the effects of growth rate and ontogeny on the otolith size – fish size relationship were evaluated in terms of their potential role in affecting the proportionality of otolith growth and somatic growth. Finally, these findings were considered in terms of available back-calculation techniques. The overall intent was to choose an appropriate procedure for back-calculation, one that best accounts for the complexities of the otolith size – fish size relationship.

As stated above, back-calculation assumes that otolith growth and somatic growth are in constant proportion. Studies examining otolith and somatic growth, however, have used the terms coupling and decoupling. In this paper, proportional growth will be used in place of the term coupling. By extension, if otolith growth and somatic growth are not in constant proportion, then this equates with the term decoupling. This terminology will be used because the assumptions of back-calculation are phrased in terms of proportionality. There is also a large body of literature examining the general relationship between the growth of various body parts (see reviews of allometry by Cock 1966; Gould 1966). The continued use of the terms coupling and decoupling to describe the relationship between otolith growth and somatic growth serves only to isolate this specific problem from the more general context of allometry and from the basic assumptions of back-calculation.

## Material and methods

### Larval collection and otolith procedures

The ichthyoplankton methods used for the collection of young bluefish have been described in detail elsewhere (Hare and Cowen 1991, 1994; Cowen et al. 1993). Bluefish were chosen for otolith analysis to represent the entire size range collected, from 2-mm yolk-sac larvae to 25-mm pelagic juveniles. Standard lengths of all individuals ($n = 112$) were measured to the nearest 0.01 mm using an ocular micrometer. Body depth and caudal peduncle depth were also measured to the nearest 0.01 mm with an ocular micrometer from a subset of individuals ($n = 60$) as part of another study (see Hare and Cowen 1994). From the measurements of length, body depth, and caudal peduncle depth, body area was calculated assuming a simplified fish shape (Fig. 1).

Both sagittal and lapillar otoliths were removed from all individuals for determination of age and otolith size. The otoliths to be used in this study were selected on the basis of an initial evaluation of otolith clarity. Otoliths were examined at 400× magnification on at least three separate occasions in random order with the aid of an image analysis system. Each examination included enumeration of the number of daily increments and measurement of the maximum otolith radius. Nyman and Conover (1988) determined that sagittal increments are formed daily in estuarine juvenile bluefish and Hare and Cowen (1994) argued that sagittal increments are also formed daily in larval and pelagic juvenile bluefish. Whether increment formation in the lapillus is daily has been questioned for preflexion larvae (Hare and Cowen 1994), and thus age was estimated by calculating the mean number of sagittal increments for each individual. Likewise, maximum otolith radius was estimated by calculating the mean value from each examination of an individual otolith.

The ontogenetic stage of each individual was determined on the basis of otolith characters previously described (Hare and Cowen 1994). Hare and Cowen (1994) found that (*i*) the first otolith increment forms at hatching, (*ii*) there is an apparent change in the increment deposition rate of the lapillus associated with flexion, and (*iii*) secondary growth centers begin to form at the transition from larva to juvenile. On the basis of these findings, individuals with two or fewer sagittal increments were classified as yolk-sac larvae ($n = 32$). Individuals with a lapillus/sagitta increment ratio $> 1.75$ were classified as preflexion larvae ($n = 24$) (see Fig. 2). If the lapillus/sagitta ratio was $\leq 1.75$, individuals were classified as postflexion ($n = 38$) (see Fig. 2). Individuals with secondary growth centers present on their sagitta were termed posttransformation pelagic juveniles ($n = 18$).

### Statistical background and analyses

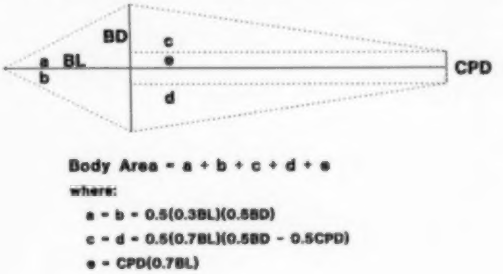
The basic approach used to examine the age-independent variability in the otolith size – fish size relationship was to model otolith size and fish size with regard to age. The residuals of these models were then used to quantify the amount of age-independent variability. A fish with a positive fish size-on-age residual represents a fish that is large for its age. A fish with a positive otolith size-on-age residual represents a fish with a large otolith for its age. If there is no age-independent variability in the otolith size – fish size relationship, then the residuals of the two models (otolith size-on-age and fish size-on-age) should be perfectly correlated (Fig. 3A). By extension, a decreasing correlation between otolith size-on-age and fish size-on-age residuals equates to increasing age-independent variability.

Two factors that may affect the proportionality of otolith and somatic growth were examined: growth rate effects and ontogenetic effects. To examine growth rate effects, an approach similar to the analysis of age-independent variability was used. Otolith size and fish age were modeled with regard to fish size. A fast-growing fish will have a negative residual; it will be young for its size. If fast-growing fish have smaller otoliths, then the residuals of age-on-fish size and otolith size-on-fish size will be positively correlated (Fig. 3B), thereby demonstrating a growth rate effect on the otolith size – fish size relationship.

The effect of ontogeny on the otolith size – fish size relationship was examined using two approaches. First, age-independent variability and growth rate effects were analyzed for specific ontogenetic stages. The results of these analyses were then compared to determine if there were ontogenetic differences. Second, the slopes of the otolith size – fish size relationship for the various ontogenetic stages were compared. If the slopes are significantly different, then otolith growth is not in constant proportion with somatic growth (Cock 1966).

To conduct the analysis of residuals, several different relationships were modeled: fish size-on-age and otolith size-on-age (for the analysis of age-independent variability), as well as age-on-fish size and otolith size-on-fish size (for the analysis of growth rate effects). Both sagittae and lapilli were examined to test whether the results of the various analyses differed between otoliths. In addition, two measures of fish size were used to test whether the measure of body size affected the age-independent variability in the otolith size – fish size relationship. Length was measured in all individuals and body area was calculated from a subset of larvae. Thus, a total of seven combinations of variables were modeled for the analyses of residuals: length-on-age, body area-on-age, sagittal radius-on-age, lapillar radius-on-age, age-on-length, sagittal radius-on-length, and lapillar radius-on-length.

**Fig. 1.** Schematic of method by which body area of young bluefish was calculated. A simplified fish shape was used where BD is body depth (mm), BL is body length (mm), and CPD is caudal peduncle depth (mm).


BD
c
a
BL
e
b
d
CPD
Body Area = a + b + c + d + e
where:
a = b = 0.5(0.3BL)(0.5BD)
c = d = 0.5(0.7BL)(0.5BD – 0.5CPD)
e = CPD(0.7BL)

The analyses were conducted in several steps. (*i*) Since a residual as a measure of an individual's departure from the population is dependent on the model used, a variety of models were fit to the various relationships (e.g., length-on-age). (*ii*) The best model for a given relationship was then chosen on the basis of several criteria. (*iii*) Residuals from the best models in terms of age were analyzed to determine the age-independent variability in the otolith size – fish size relationhip. (*iv*) Residuals from the best models in terms of length were analyzed to determine the effect of growth rate on the otolith size – fish size relationship. (*v*) Ontogenetic effects were evaluated by testing for differences between stages in age-independent variability and growth rate effects, and the slopes of the otolith size – fish size relationship were compared between the different ontogenetic stages.

Explaining these steps in more detail, the first step applied first- through fourth-order polynomial models to each of the seven combinations of variables. Campana and Jones (1992) have discussed the use of linear and curvilinear functions for modeling fish growth. It is important to note that these models are used solely to develop the best fit for the seven combinations of variables from which the residuals will be used in the analysis of age-independent variability and of growth rate and ontogenetic effects. Each of the seven combinations of variables was modeled using the following eight equations:

$y = a + bx$ model 1
$y = a + bx + cx^2$ model 2
$y = a + bx + cx^2 + dx^3$ model 3
$y = a + bx + cx^2 + dx^3 + ex^4$ model 4
$\ln(y) = a + bx$ model 5
$\ln(y) = a + bx + cx^2$ model 6
$\ln(y) = a + bx + cx^2 + dx^3$ model 7
$\ln(y) = a + bx + cx^2 + dx^3 + ex^4$ model 8

**Fig. 2.** Schematic representation of method by which pre- and post-flexion larvae were distinguished on the basis of lapillar and sagittal increment number. Figurative data are the lapillar increment number for three illustrative individuals, with the ratio of sagittal to lapillar increments given in parentheses. Hare and Cowen (1994) found an apparent change in the increment formation rate of the lapillus at flexion from one increment every other day to one increment every day. Given this pattern, the relative number of sagittal to lapillar increments defines individuals as pre- or post-flexion. Increment at hatching is designated number 0, so that sagittal increment number represents age. Yolk adsorption occurs at about 2 days (Hare and Cowen 1994). The ratio of sagittal and lapillar increments decreasing to 1.75 is indicative of the change in increment deposition rate and thus the occurrence of flexion.

| | Sagittal Increment Number | | | | | | | | | | | | | | | Individual |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| Lapillar Increments | | 1<br>(2.00) | | 2<br>(2.00) | | 3<br>(2.00) | | 4<br>(2.00) | | 5<br>(2.00) | | 6<br>(2.00) | 7<br>(1.85) | 8<br>(1.75) | 9<br>(1.67) | 1 |
| | | 1<br>(2.00) | | 2<br>(2.00) | | 3<br>(2.00) | | 4<br>(2.00) | | 5<br>(2.00) | 6<br>(1.83) | 7<br>(1.71) | 8<br>(1.63) | 9<br>(1.56) | 10<br>(1.50) | 2 |
| | | 1<br>(2.00) | | 2<br>(2.00) | | 3<br>(2.00) | | 4<br>(2.00) | 5<br>(1.80) | 6<br>(1.67) | 7<br>(1.57) | 8<br>(1.50) | 9<br>(1.44) | 10<br>(1.40) | 11<br>(1.36) | 3 |
| Ontogenetic Stage | Yolk-Sac Larvae | | Pre-Flexion Larvae | | | | | | | Post-Flexion Larvae | | | | | | |

where *y* is the dependent variable; *x* is the independent variable; and a, b, c, d, and e are constants of a given model. Models were fit to the data using the multiple general linear hypotheses module of SYSTAT (Wilkinson 1990).

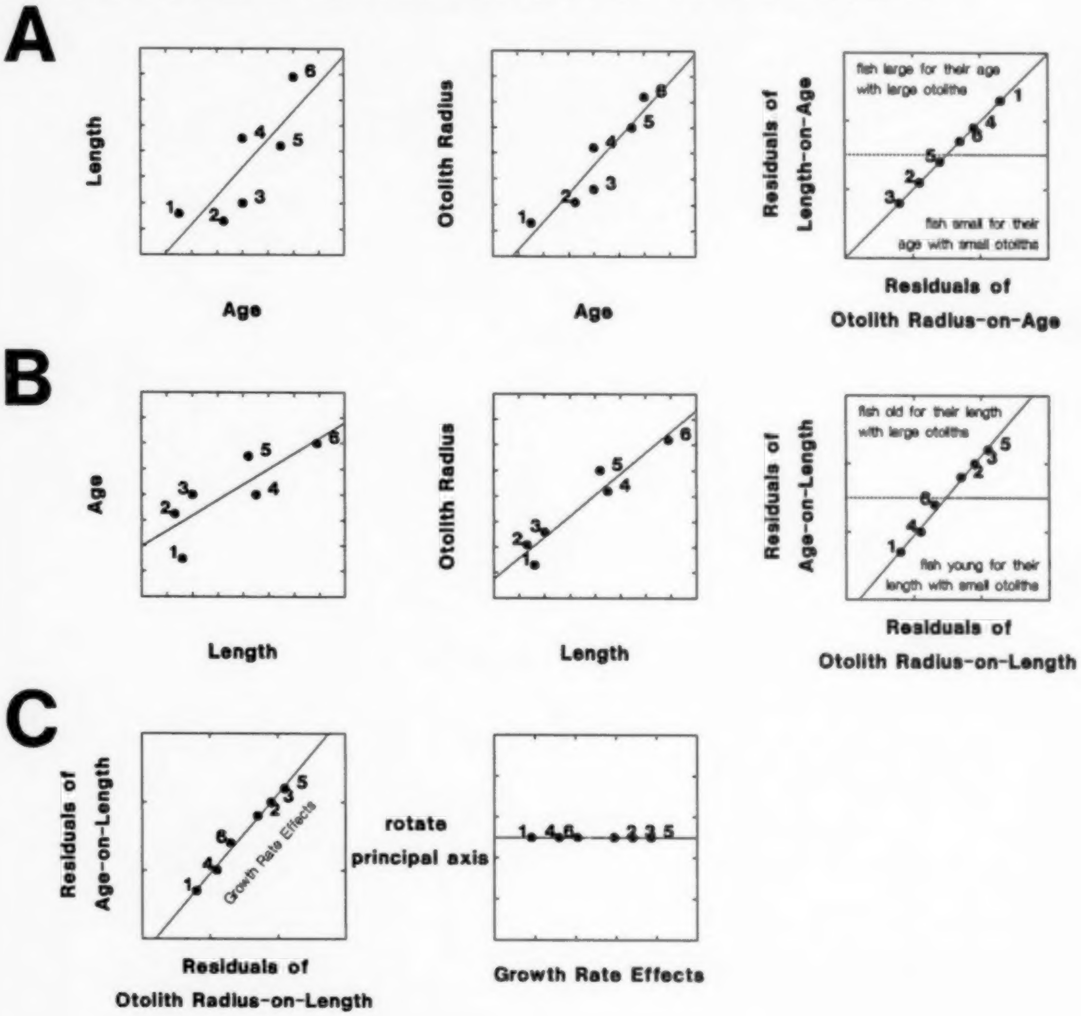
The second step determined which of the eight models best fit each of the combination of variables. This determination was based on several criteria common to linear regression analyses. Specifically, the residuals of the model must be normally distributed, they must have a constant variance with respect to the independent variable, and they must be independent of each other. In addition, all data in the relationship must be described by the same model, and the model must explain a significant amount of the relationship's variation. These conditions were evaluated for each model, and then the model with the fewest parameters, which best satisfied these criteria, was used to calculate the residuals of a given relationship for further analysis. Normality of a model's residuals was tested using a Kolmogorov–Smirnov test ($\alpha = 0.05$). Constant variance and independence of a model's residuals were evaluated visually by plotting residuals versus predicted values. Whether all values of a given relationship were adequately described by the model was quantified by counting the number of residuals with Cook's distance values >0.5. Cook's distance measures the influence of each observation on the model's coefficients. A large Cook value means that if that observation were removed, the coefficients of the model would change substantially. The *F* statistic and $r^2$ value were used as relative measures of the significance of a given model. All tests and analyses were performed using SYSTAT and are described in Wilkinson (1990).

Once a model was chosen for each relationship, the third step examined the age-independent variability in the relationship between otolith size and fish size. Pearson correlation coefficients (Sokal and Rohlf 1981) were calculated of the residuals from the otolith size-on-age models and the body size-on-age models. Differences between the measure of body size and otolith size were then evaluated using a test of homogeneity among correlation coefficients outlined in Sokal and Rohlf (1981) with a significance level of $\alpha = 0.05$. The amount of age-independent variability is the amount of variability in the body size-on-age residuals that is not explained by otolith size-on-age residuals.

The fourth step analyzed the effect of growth rate on the otolith size – fish size relationship. Pearson correlation coefficients were calculated of the otolith radius-on-length models and the age-on-length model residuals. Growth rate effects were then quantified through an analysis of the principal axes (see Sokal and Rohlf 1981) of the otolith radius-on-length residuals and the age-on-length residuals. The principal axis in this case represented the axis of slower growing fish with larger otoliths and faster growing fish with smaller otoliths. The residuals were rotated so that the principal axis became the *x* axis. In this orientation, the *x* value represents the degree to which an individual's otolith size was affected by differences in growth rate (Fig. 3C). This estimate of growth rate effects was then used in a multiple regression model with the residuals of otolith size-on-age to determine how much variability in the body size-on-age residuals could be explained, effectively accounting for age and growth rate effects on the otolith size – fish size relationship. The multiple general linear hypotheses module of SYSTAT (Wilkinson 1990) was used.

The first part of the final step repeated the above analyses for the different ontogenetic stages: yolk-sac, larvae,

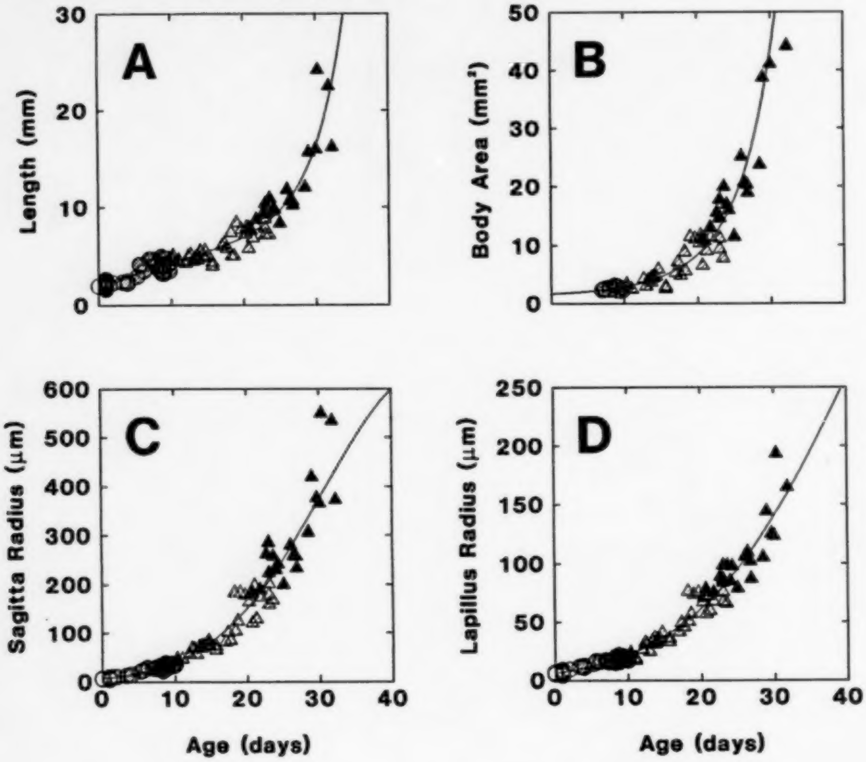
**Fig. 3.** Schematic of the predictions that (A) fish larvae large for their age will have large otoliths for their age (to determine age-independent variability) and (B) fish larvae old for their length will have large otoliths for their length (to determine growth rate effects), and (C) method by which growth rate effects were quantified.



preflexion larvae, postflexion larvae, and pelagic juveniles. Pearson correlation coefficients were calculated for the relationship between otolith size-on-age residuals and length-on-age residuals for each ontogenetic stage (the analysis of age-independent variability). Pearson correlation coefficients were also calculated for each ontogenetic stage for the relationship between otolith radius-on-length residuals and age-on-length residuals (the analysis of growth rate effects). Comparisons of stage-specific correlation coefficients were made using a pairwise test of homogeneity among two coefficients (Sokal and Rohlf 1981). Since all comparisons were made, a Bonferroni correction was used to determine the level of significance; for each analysis, six comparisons were made resulting in a Bonferroni significance level of $\alpha = 0.008$ (Wilkinson 1990). The multiple regression, which included the rotated axis of the growth rate effect, was also repeated for each ontogenetic stage. Multiple correlation coefficients ($r$ values) derived from the model of each ontogenetic stage were then compared with the multiple correlation coefficient derived from the model that included all stages (from above) using the test of homogeneity among correlation coefficients (Sokal and Rohlf 1981). Since specific tests were planned (model $r$ value of each stage to model $r$ value of all stages), a Bonferroni correction was not used and significance was tested at $\alpha = 0.05$.

The final portion of the fifth step calculated the slope of the otolith size – fish size relationship for each ontogenetic stage. The equation of simple allometry was used, $y = ax^k$, where the log transformation results in $\log(y) = \log(a) +$

**Fig. 4.** Data and best-fit models for (A) length-on-age, (B) body area-on-age, (C) sagittal radius-on-age, and (D) lapillar radius-on-age relationships for young bluefish. Ontogenetic stages are distinguished by different symbols: open circles are yolk-sac larvae, hatched circles are preflexion larvae, hatched triangles are postflexion larvae, and solid triangles are pelagic juveniles.



$k\log(x)$ (Gould 1966). If otolith and somatic growth are in constant proportion throughout the ontogeny of bluefish, then the slope ($k$) should not be different between ontogenetic stages. All slopes were compared using an unplanned comparison of a set of regression coefficients (Sokal and Rohlf 1981).

## Results

### Model choice

Otolith size-on-age relationships and body size-on-age relationships were described best by second- and third-order natural log-transformed models, respectively. For length-on-age, the third-order natural log transformed model was chosen (model 7; Fig. 4A), because of the independence of its residuals and the higher $r^2$ value compared with the first- and second-order models. The second-order natural log transformed model was chosen for the body area-on-age relationship (model 6; Fig. 4B), because of the independence and constant variance of the residuals and the higher $r^2$ value. The second-order natural log transformed model was also chosen for sagittal radius-on-age and lapillar radius-on-age relationships (model 6; Figs. 4C and 4D). In the case of sagittal radius-on-age, this model was chosen because of the independence of the residuals and the higher $F$ statistic and $r^2$ value; in the case of lapillar radius, this model was chosen because of the normality and independence of the residuals, as well as the higher $r^2$ value.

Third-order models were chosen for the age-on-length and otolith radius-on-length relationships. The nontransformed third-order model was chosen for the age-on-length relationship (model 3; Fig. 5A), because of independence of the residuals and Cook's distance values. However, the natural log transformed third-order model was chosen for both the sagittal radius-on-length and lapillar radius-on-length relationships (model 7; Figs. 5B and 5C), because of the normality and independence of residuals and higher $F$ statistic and $r^2$ values.

### Age-independent variability

Analysis of otolith size-on-age and body size-on-age residuals demonstrated that there is variability in the otolith size – fish size relationship when the effect of age is removed. Pearson correlation coefficients for length and otolith radius residuals were 0.639 ($p < 0.001$) and 0.593 ($p < 0.001$) for the sagitta and lapillus, respectively (Figs. 6A and 6B). Pearson correlation coefficients for body area and otolith radius residuals were 0.840 ($p < 0.001$) and 0.722 ($p < 0.001$)

for the sagitta and lapillus, respectively (Figs. 6C and 6D). The correlation coefficient for the sagittal radius – body area residuals was significantly greater than the coefficient for the sagittal radius – body length residuals ($t_s = 2.853$, $p < 0.01$), while the coefficients for the lapillar radius – body area and lapillar radius – body length residuals were not significantly different ($t_s = 1.411$, $p > 0.05$).

### Growth rate effects

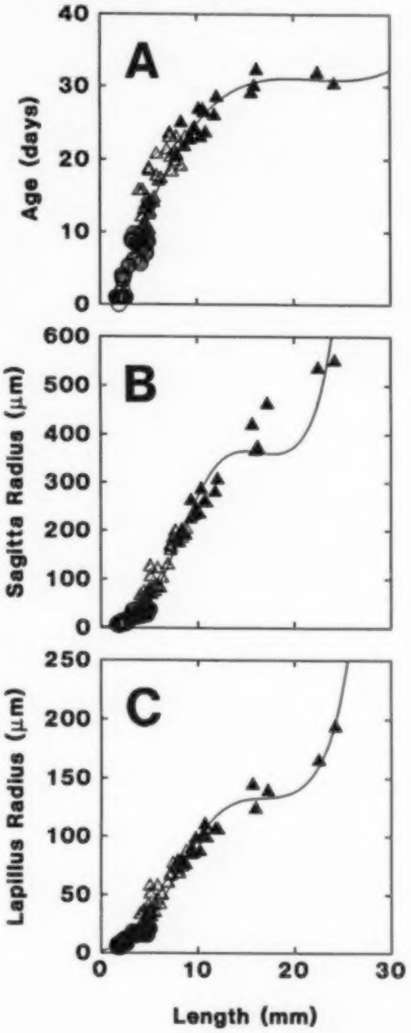
Comparison of the residuals of age-on-length with the residuals of otolith radius-on-length demonstrated that there was a significant effect of growth rate on the otolith size – fish size relationship. Pearson correlation coefficients were 0.807 ($p < 0.001$) and 0.770 ($p < 0.001$) for the sagitta and lapillus, respectively (Figs. 7A and 7B). When the rotated principal axes of these relationships (i.e., the measure of the effect of growth rate) were included in a multiple regression model with the residuals of otolith radius-on-age, a significant amount of the variation in the length-on-age residuals was explained ($r^2 = 0.800$ and 0.830, for sagitta and lapillus, respectively, $p < 0.001$ for both otoliths). Thus, if growth rate effects are accounted for, much of the age-independent variation in fish size can be explained by otolith size.

### Ontogenetic effects

The stage-specific analysis of age-independent variability found differences between ontogenetic stages. Age-independent variability decreased from the preflexion stage to the pelagic juvenile stage (Table 1). With regard to sagittal radius and length, there was a significantly greater correlation between otolith size-on-age and length-on-age residuals in the postflexion stage than in the preflexion stage ($t_s = 3.438$, $p < 0.001$, Bonferroni correction $\alpha = 0.008$). In terms of lapillar radius and length, there was a significantly greater correlation between otolith size-on-age and length-on-age residuals in the pelagic juvenile stage than in the preflexion stage ($t_s = 3.085$, $p < 0.008$, Bonferroni correction $\alpha = 0.008$) and the postflexion stage ($t_s = 2.822$, $p < 0.008$, Bonferroni correction $\alpha = 0.008$). Other comparisons were not significant.

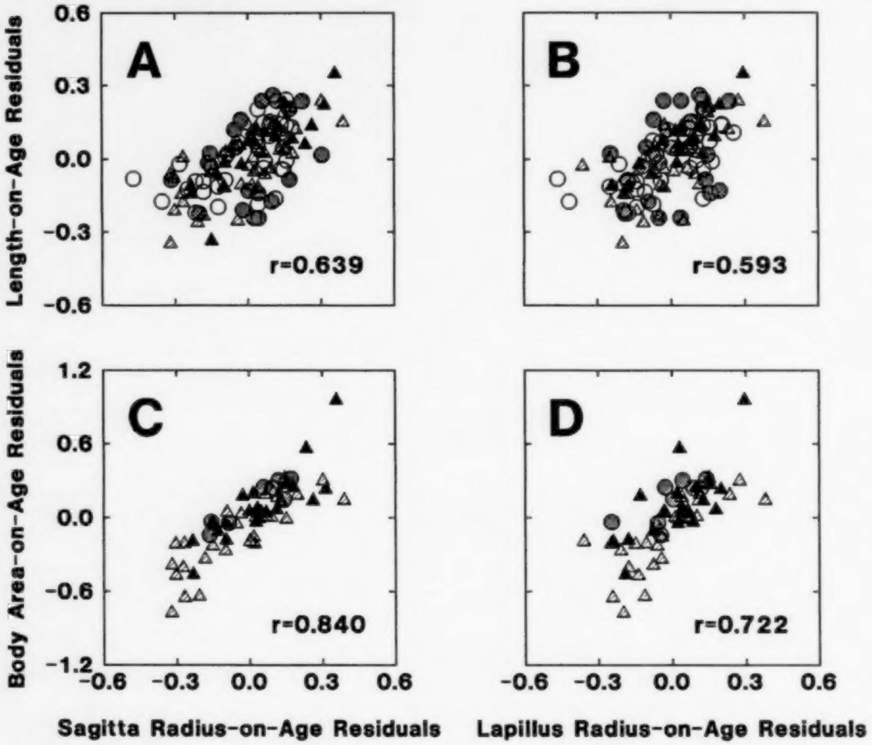
Ontogenetic differences were also found in the effect of growth rate on the otolith size – fish size relationship (Table 1). With regard to the sagitta, growth rate effects were significantly lower in pelagic juveniles than in preflexion larvae ($t_s = 4.060$, $p < 0.001$, Bonferroni correction $\alpha = 0.008$) and postflexion larvae ($t_s = 3.673$, $p < 0.001$, Bonferroni correction $\alpha = 0.008$). Growth rate effects were significantly higher in preflexion larvae than in yolk-sac larvae ($t_s = 3.240$, $p < 0.001$, Bonferroni correction $\alpha = 0.008$) and postflexion larvae ($t_s = 2.941$, $p < 0.008$, Bonferroni correction $\alpha = 0.008$) (Table 1). In terms of the lapillus, growth rate effects were significantly lower in both yolk-sac larvae and pelagic juveniles than in preflexion larvae ($t_s = 3.965$, $p < 0.001$ and $t_s = 3.192$, $p < 0.001$, respectively, Bonferroni correction $\alpha = 0.008$) and postflexion larvae ($t_s = 3.510$, $p < 0.001$ and $t_s = 3.320$, $p < 0.001$, respectively, Bonferroni correction $\alpha = 0.008$). Other comparisons were not significant. Thus, decreases in the effect of growth rate coincide with decreases in the amount of age-independent variability in the otolith size – fish size relationship (Fig. 8A).

**Fig. 5.** Data and best-fit models for (A) age-on-length, (B) sagittal radius-on-length, and (C) lapillar radius-on-length relationships for young bluefish. Ontogenetic stages are represented as in Fig. 4.



When growth rate effects (the rotated principal axis of otolith radius-on-length residuals and age-on-length residuals; see Fig. 3C) were incorporated into stage-specific multiple regression models with the residuals of otolith radius-on-age, more of the variability in length-on-age residuals was explained compared with the models that combined all ontogenetic stages (Table 2). Multiple correlation coefficients for the yolk-sac and postflexion larvae were significantly greater than those of the general models for both otoliths. In addition, the explanatory power of these multivariate models apparently decreased during development, suggesting an increase in the variability of the otolith size – fish size relationship that is not due to age differences or growth rate effects (Fig. 8B).

**Fig. 6.** Correlation analysis of residuals evaluating amount of age-independent variability in the otolith size – fish size relationship of young bluefish: (A) length versus sagittal radius residuals, (B) length versus lapillar radius residuals, (C) body area versus sagittal radius residuals, and (D) body area versus lapillar radius residuals. The greater the correlation, the less the age-independent variability (see Fig. 3A). Ontogenetic stages are represented as in Fig. 4.



Ontogenetic differences were also found between the stage-specific slopes of the simple allometric approximation of the otolith size – fish size relationship (Fig. 9). For the sagitta, the slope for the postflexion stage was significantly greater than that of the other three stages (difference = 1.187, $p < 0.001$; 1.112, $p < 0.001$; 1.096, $p < 0.001$ for yolk-sac larvae, preflexion larvae, and pelagic juveniles, respectively). Other comparisons were not significant. Similarly, for the lapillus, the slope for the postflexion stage was significantly greater than that for the other three stages (difference = 0.801, $p < 0.05$; 1.224, $p < 0.001$; 1.018, $p < 0.001$; for yolk-sac larvae, preflexion larvae, and pelagic juveniles, respectively). Other comparisons were not significant.

## Discussion

### Proportionality of the otolith size – fish size relationship

The results of this study demonstrate that there is age-independent variability in the otolith size – fish size relationship (Fig. 6) and that this variability is stage specific (Fig. 8A). The analyses of the effect of growth rate revealed that much of the age-independent variation could be accounted for by differences in growth rate, but again this was shown to be stage specific (Fig. 8A). If ontogenetic effects and growth rate effects were both accounted for, then a greater amount of the variability in fish size could be explained (Table 2), but unexplained variability increased ontogenetically (Fig. 8B). Since it is characteristic for young fish to have variable growth rates and to undergo ontogentic stage transitions, a back-calculation technique should be used that can account for both growth rate and ontogenetic effects on the otolith size – fish size relationship. However, before back-calculation methods are considered, the factors that may affect the proportionality of otolith growth and somatic growth must be discussed in more detail.

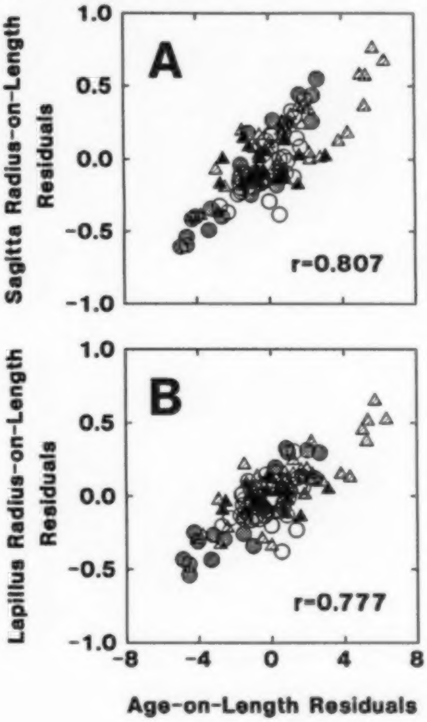
There are several factors that may affect the otolith size – fish size relationship, as well as the relationship between otolith growth and somatic growth. One factor identified in this study is the measure of somatic size used. Many studies base their acceptance or rejection of proportionality between otolith growth and somatic growth on a correlation between otolith size and fish size. In this study, less age-independent variability was found between body area and otolith radius than between length and otolith radius. Thus, in a given study, the measure of body size used may not be the measure that is most closely correlated

**Table 1.** Pearson correlation coefficients calculated for length-on-age residuals and otolith radius-on-age residuals (analysis of age-independent variability) and for age-on-length residuals and otolith radius-on-length residuals (analysis of growth rate effects) for each ontogenetic stage.

| | Correlations between residuals of length-on-age and otolith radius-on-age | | Correlations between residuals of age-on-length and otolith radius-on-length | |
|---|---|---|---|---|
| Ontogenetic stage | Sagittal radius | Lapillar radius | Sagittal radius | Lapillar radius |
| Yolk-sac larvae | 0.632*** | 0.663*** | 0.627*** | 0.264*** |
| Preflexion larvae | 0.340 | 0.330 | 0.931*** | 0.887*** |
| Postflexion larvae | 0.733*** | 0.611*** | 0.824*** | 0.818*** |
| Pelagic juveniles | 0.876*** | 0.919*** | 0.034 | 0.124 |

***$p < 0.001$.

**Fig. 7.** Correlation analysis of residuals evaluating the occurrence of growth rate effects in the otolith size – fish size relationship of young bluefish: (A) sagittal radius versus age residuals and (B) lapillar radius versus age residuals. The greater the correlation, the greater the effect of growth rate (Fig. 3B). Ontogenetic stages are represented as in Fig. 4.



with otolith size. Although this measurement effect is not a true biological factor, it must be recognized prior to drawing conclusions on the basis of the correlation between otolith size and fish size.

Temperature, on the other hand, may biologically affect the relationship between otolith and somatic growth. Mosegaard et al. (1988) found that otolith growth responded differently to temperature than somatic growth; at temperatures beyond those typically encountered by the organism (hyperoptimal), somatic growth decreased while otolith growth increased. These results demonstrate that the proportionality between otolith and somatic growth may be temperature dependent and, therefore, not constant.

Another factor that may affect the relationship between otolith growth and somatic growth is differences in the response time of somatic and otolith growth to changes in the environment. Molony and Choat (1990) were able to distinguish somatic size differences in experimental populations prior to recognizing otolith size differences and they concluded that there is a lag in the response of otolith growth compared with somatic growth. Gutiérrez and Morales-Nin (1986) found that increment width and temperature were cross-correlated with lags of 0–7 d, suggesting that otolith growth is lagged from changes in temperature, which by extension could create changes in somatic growth. Thus, otolith growth and somatic growth may respond to changes in the environment (e.g., ration level, temperature) at different rates, indicating that during such periods, the relationship between otolith growth and somatic growth cannot remain constant.

In addition to differences in response time, a variety of studies have shown that otolith growth continues during periods of no somatic growth, as well as during periods of somatic loss (Marshall and Parker 1982; Volk et al. 1984; Eckmann and Rey 1987; Maillet and Checkley 1990; Sogard 1991). These observations indicate that there is some minimal rate of otolith growth, which is independent of somatic growth. Secor and Dean (1989) argued that otolith growth continues during periods of no somatic growth because of the incremental nature of otolith increment deposition. Positive minimal otolith growth during periods when somatic size is not increasing or is decreasing implies that there is not a simple proportional relationship between otolith growth and somatic growth (i.e., the relationship is not $dO/dA = i(dL/dA)$, where $O$ is otolith radius, $L$ is fish length, $A$ is age, and $i$ is a coefficient of proportionality).

The relationship between otolith growth and somatic growth is also affected by the absolute growth rate. Many studies have found that faster growing fish have smaller otoliths at a given length than slower growing fish (i.e., a growth rate effect on the otolith size – fish size relationship) (e.g., Templeman and Squires 1956; Reznick et al. 1989; Secor and Dean 1989; Hovenkamp 1990; Wright et al. 1990; Francis et al. 1993; the present study). If an individual's growth rate relative to the population's average growth rate does not change, then the assumption of proportionality is not violated (Campana 1990). For example, in the simple linear case, the assumption of scale proportional back-calculation (i.e., if a scale were 10% larger at capture than the average scale of a fish of comparable size, then the scale would be 10% larger than average throughout

**Fig. 8.** (A) Correlation coefficients from the analyses of age-independent variability and growth rate effects. Age-independent variability was estimated by subtracting the correlation coefficient of length-on-age and otolith size-on-age residuals from 1 (see Figs. 3A and 6). Growth rate effects were estimated by using the correlation between otolith size-on-length and age-on-length residuals (see Figs. 3B and 7). Comparisons for both sagitta and lapillus are distinguished by S and L, respectively. Ontogenetic stages are represented as in Fig. 4. (B) Correlation coefficients of the multiple regression models for both otoliths (distinguished by S and L as above) and each ontogenetic stage ($x$ axis): yolk-sac larvae (YSL), preflexion larvae (PreFL), postflexion larvae (PFL), and pelagic juveniles (PJ).

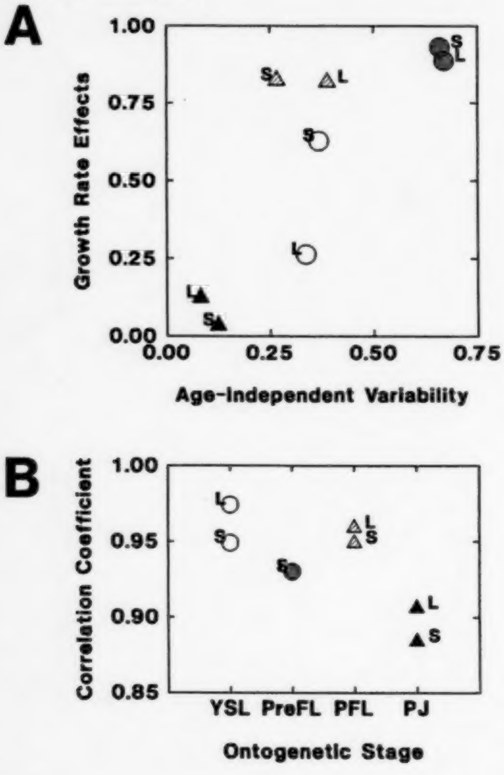
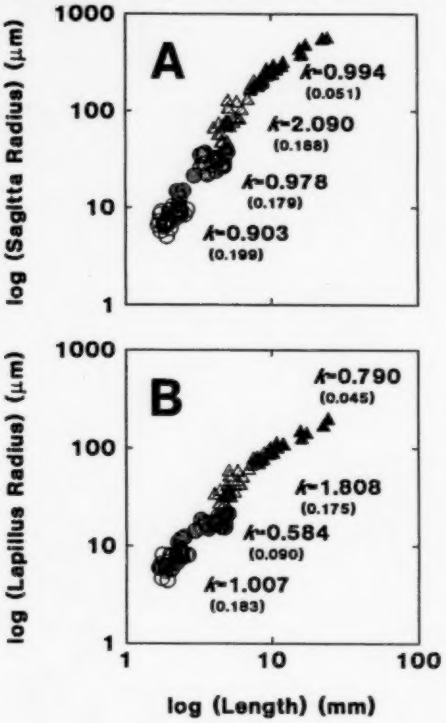


**Fig. 9.** Simple allometric plot of the (A) sagittal radius–length and (B) lapillar radius–length relationship for young bluefish. Slopes of the equation log(otolith radius) = log(b) + $k$log(length) for each ontogenetic stage are provided, and the standard error of the slopes are in parentheses. Ontogenetic stages are represented as in Fig. 4.



the life of the fish) can be interpreted as a growth rate effect; a slower growing fish will have a larger scale at a given length than a faster growing fish. Campana (1990) demonstrated, however, that as the effect of growth rate increases (i.e., slower growing fish have increasingly larger otoliths at a given size), the potential for back-calculation error increases. Also, if an individual's growth rate relative to the population's growth rate changes (e.g., as observed in salmon fry by Wilson and Larkin 1982), then the assumption of constant proportionality will be violated.

Finally, there are two types of ontogenetic influences that may affect the relationship between otolith growth and somatic growth. This study found that the age-independent variability in the otolith size – fish size relationship and the effect of growth rate on this relationship differed between ontogenetic stages (Fig. 8). The sources of these differences remain unclear, yet this finding implies that the accuracy of any back-calculation procedure may be stage specific.

A second ontogenetic effect is the occurrence of stage-specific otolith growth – somatic growth relationships (Campana 1984; Rice et al. 1985; Cowen 1991; Toole et al. 1993; Hare and Cowen 1994; the present study). Campana (1990) modeled an ontogenetic change in growth rates using size to determine the timing of a life history transition and found that his biological intercept procedure (a modified proportional technique) back-calculated fish sizes accurately. Laidig et al. (1991) used a curvilinear proportional technique to back-calculate fish size, with inflection points in essence representing ontogenetic changes in the otolith size – somatic size relationship. Life history stage transitions, however, occur at variable size and age (Chambers and Leggett 1987, 1992; Cowen 1991; McCormick 1994; Sponaugle and Cowen 1994) and thus ontogenetic changes will result in a complex relationship between otolith size and fish size. More importantly, these changes imply that the relationship between otolith growth and somatic growth is not constant through life history stage transitions (Fig. 9), thereby violating the assumptions of proportional back-calculation.

It is clear that many factors affect the relationship between otolith growth and fish growth and that additional research is necessary to elucidate this relationship. This study involved a cross-sectional analysis of the otolith size – fish size relationship for field-collected bluefish larvae and pelagic juveniles (i.e., one set of measurements per individual with individuals representing different ages and ontogenetic stages; see Cock 1966). Other studies have performed cross-sectional analyses on controlled populations (e.g., Secor and Dean 1989, 1992). To further our understanding of the functional relationship between otolith growth and somatic growth, longitudinal studies are necessary. The size of individuals must be tracked over time and this individual record of size must be compared with the size record contained within the individual's otolith (see Cock 1966). Several studies have followed individuals over time (e.g., Wilson and Larkin 1982), and these should be used as a foundation for more involved work. A multivariate approach should be used to estimate fish size and otolith size, since the first component in a multigroup principal components analysis can be a consistent measure of general size (Strauss and Fuiman 1985; Lombarte and Lleonart 1993). Treatments should examine the different factors that affect the otolith growth – somatic growth relationship: temperature, growth rate, response lags, and ontogenetic transitions. Such a set of experiments would allow a comprehensive analysis of the functional relationship between otolith and fish growth and a thorough evaluation of the appropriateness of the various back-calculation procedures.

This study, however, provides evidence that a basic assumption of proportional back-calculation may be violated in the early life history stages of fishes. Ontogenetic changes in the relationship between otolith size and somatic size imply that there are ontogenetic changes in the relationship between otolith growth and somatic growth (see Cock 1966; Gould 1966; Laird et al. 1968; Shea 1985). In addition, if individual growth rates change relative to the population's growth rate, then as a result of growth rate effects, the proportionality between otolith growth and somatic growth must necessarily change (see Campana 1990). This study indicates, however, that if growth rate effects and ontogenetic effects can be accounted for in back-calculation, then much of the variability in the otolith size – fish size relationship can be explained (Table 2).

### Back-calculation and fish early life history stages

Two potential approaches exist by which growth and ontogenetic effects can be incorporated into existing back-calculation procedures for the estimation of size in the early life history stages of fishes. The first is to use stage-specific multiple regression of otolith size and fish age to estimate fish size. The idea that age be included in a regression back-calculation formula to account for growth rate effects was suggested by Secor and Dean (1992). Ontogenetic effects would be accounted for by using a different multiple regression model for each ontogenetic stage. The second approach is to modify Campana's (1990) biological-intercept procedure and use stage-specific otolith growth – somatic growth relationships to incorporate ontogenetic effects. Age could also be incorporated to correct for growth rate effects. This model would be similar to Campana's (1990) eq. 7, which used a series of daily growth increments to compensate for time-varying growth rates. Both of these approaches would require that the timing of ontogenetic transitions be determined from the otolith record (see Hare and Cowen 1994), so that the correct regression or otolith growth – somatic growth proportion could be applied to an otolith size in a given ontogenetic stage. It is important to note that incorporating these changes does not deal with temperature-derived effects and response time effects on the otolith growth – somatic growth relationship.

**Table 2.** Multiple correlation coefficients for multiple regression models (length-on-age residuals = constant + otolith radius-on-age residuals + principal axis of growth rate effects).

| | Sagittal radius | | Lapillar radius | |
|---|---|---|---|---|
| Ontogenetic stage | *r* | $t_s$ | *r* | $t_s$ |
| All stages | 0.894 | — | 0.911 | — |
| Yolk-sac larvae | 0.949 | −3.951*** | 0.974 | −3.024*** |
| Preflexion larvae | 0.930 | −0.908 | 0.930 | −0.525 |
| Postflexion larvae | 0.949 | −1.959* | 0.959 | −2.063 |
| Pelagic juveniles | 0.884 | 0.180 | 0.906 | 0.102 |

**Note**: The $t_s$ values are provided for comparisons between stage-specific models and the model for all ontogenetic stages.

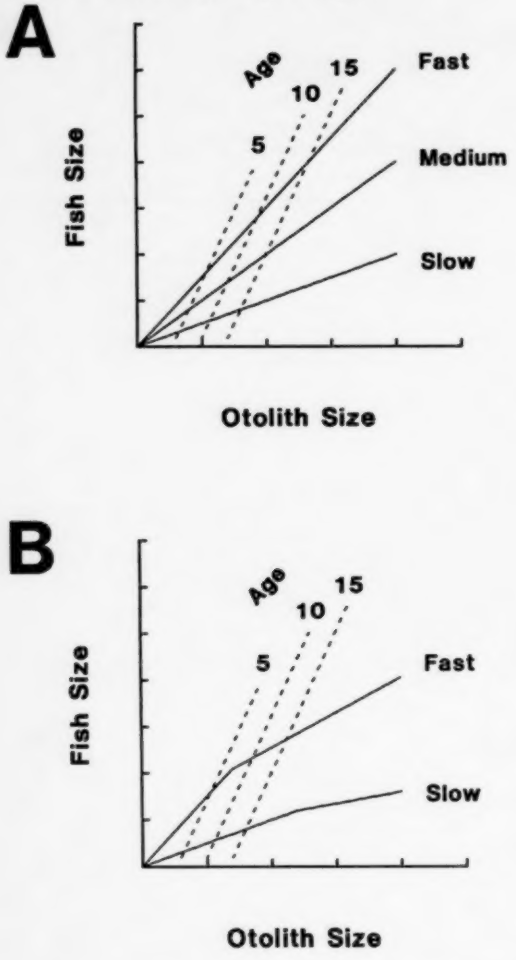
*$p < 0.05$.

***$p < 0.001$.

The use of regression techniques versus proportional techniques is a topic of ongoing debate. Secor and Dean (1992) have advanced the use of regression techniques, while Francis (1990) and Campana (1990) have advanced the use of proportional techniques. Within proportional techniques, there is also a debate as to which regression should be used to estimate the parameters of the average fish (see Francis 1990; Ricker 1992) and this argument extends to other uses of linear regression (Ricker 1973; Laws and Archie 1981; Campana and Jones 1992). This study and the above discussion indicate that the basic assumption of proportional techniques may be violated by growth rate effects and by ontogenetic effects in the early life history stages of fishes. Clearly, caution should be used in the application of current back-calculation procedures to larval and juvenile fishes. In the future, longitudinal studies should be able to isolate and quantify the effects of temperature, response times, growth rate, and ontogeny on the relationship between otolith growth and somatic growth. Changes in the current back-calculation techniques, such as using a stage-specific multiple regression or a stage-specific biological intercept procedure, can then be considered in light of these new data, thereby further refining the ability to numerically estimate fish size from otolith size.

Not all back-calculation, however, requires the actual estimation of fish size from otolith size. Some questions are comparative and simply require calculating the relative fish size-at-age (see parallel approach presented by Hoenig et al. 1990). Formally stated, this relative size approach assumes that at a specific age, a larva with a larger otolith

**Fig. 10.** (A) Schematic of relative size approach of estimating relative fish size-at-age from otolith size-at-age. (B) Schematic showing that approach is not necessarily influenced by ontogenetic changes in the relationship between otolith growth and somatic growth (transition indicated by change in slope).



also has a larger body size. This statement is supported by this study and by many others (e.g., Wilson and Larkin 1982; Volk et al. 1984; Eckmann and Rey 1987; Alhossaini and Pitcher 1988; Hovenkamp 1990; Maillet and Checkley 1990; Sogard 1991; Bradford and Geen 1992; Tzeng and Yu 1992; Zhang and Runham 1992). Such a relative size approach solves the problem of growth rate effects because age is used to standardize the comparison of otolith size (Secor and Dean 1992; Fig. 10A). In addition, this method determines relative body size, so that ontogenetic stage-specific functions are not necessary to estimate actual fish size, nor is it necessary to determine the timing of life history stage transitions from the otolith record.

As discussed above, there are other factors that can influence the otolith size – fish size relationship. By comparing sizes at a specific age within a specific ontogenetic stage, stage-specific otolith growth – somatic growth relationships will not influence the relative comparisons (Fig. 10B). However, the error in such comparisons will in part be dependent on ontogenetic stage as indicated by the stage-specific differences in age-independent variability (Table 1) and by differences in the amount of variability explained by accounting for growth rate effects (Fig. 8). Response lags and temperature-mediated effects cannot be accounted for using this method and will create uncertainty, but these remain general problems common to all back-calculation. The strength of this approach is that it does not require the actual calculation of fish size, thereby removing assumptions about the specific procedures used to model the otolith size – fish size relationship. In addition, the analyses presented above allow the error in the relative size technique to be estimated for different ontogenetic stages and ages, and thus conclusions based on this technique can be evaluated with regard to this error. Finally, this method is both conceptually and practically simpler than other methods.

The relative size approach has benefits for a certain subset of back-calculation purposes, namely comparative questions. By determining the otolith size-at-age distribution of survivors (see Methot 1983; Rice et al. 1987; Crecco and Savoy 1987) and comparing this with the otolith size-at-age distribution of the larval population as a whole, the importance of size to survival can be assessed. Likewise, by comparing distributions of increment widths (e.g., the width between increment 5 and 10), patterns in survival relative to age-specific growth can be examined. However, this approach is not applicable to all comparative questions. For example, it can be used for intracohort comparisons, but for intercohort comparisons, it must first be determined that the otolith size – fish size relationship is the same among cohorts (see Secor and Dean 1989). Additionally, factors that vary both with size and age cannot be examined because of the influence of growth rate effects. For example, size-at-metamorphosis cannot be studied with the relative size approach because there is also variation in age-at-metamorphosis (Chambers and Leggett 1987, 1992; Cowen 1991; McCormick 1994; Sponaugle and Cowen 1994). Further refinements in back-calculation, such as those suggested above, are necessary before factors that confound age and size can be examined.

Back-calculation techniques provide a useful tool for the examination of the role of size and growth in the survival of larval fishes. In instances where actual numerical estimation of larval size is required, current back-calculation techniques should be used with caution because growth rate variation and ontogenetic stage transitions are characteristic of the early life history stages of fishes. The relative size approach, however, should provide a powerful method by which a variety of size- and growth-related hypotheses can be tested, thereby contributing to our understanding of the processes that create variation in larval fish survival.

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# The migratory behaviour of wild Atlantic salmon (*Salmo salar*) smolts in the estuary of the River Conwy, North Wales

**A. Moore, E.C.E. Potter, N.J. Milner, and S. Bamber**

**Abstract**: Thirty-two wild Atlantic salmon (*Salmo salar*) smolts, tagged with miniature acoustic transmitters, were tracked in the River Conwy, North Wales, to describe the freshwater and estuarine patterns of migration. Migration in fresh water was predominantly nocturnal, although there was a seasonal change in this pattern with later run fish moving during both the day and night. Smolts tagged earlier in the study spent significantly longer in the river (mean 456 ± 43 h) before migrating into coastal waters than fish tagged later in the study (mean 201 ± 30 h). The movement of smolts through the estuary was indicative of a nocturnal selective ebb tide transport pattern of migration. All of the smolts migrated seawards on an ebb tide close to the surface and within the fastest moving section of the water column. The nocturnal pattern of migration would appear to be the result of an endogenous rhythm of swimming activity that results in the smolts moving up into the water column after dusk and migrating seawards. Smolt migration in the lower portion of the estuary was indicative of active directed swimming and there was no apparent period of acclimation required when moving from fresh to salt water.

**Résumé** : Trente-deux smolts sauvages de saumon atlantique (*Salmo salar*), portant des émetteurs acoustiques miniatures, ont été pistés dans la rivière Conwy (nord du pays de Galles) en vue de la description de leurs schémas de migration en eau douce et en estuaire. La migration en eau douce était principalement nocturne, mais en observait une modification saisonnière de ce régime, les poissons des remontes tardives se déplaçant le jour comme la nuit. Les smolts marqués au début de l'étude passaient d'une période nettement plus longue dans la rivière (moyenne de 456 ± 43 h) avant d'entrer dans les eaux cotières que les poissons marqués plus tard dans l'étude (moyenne de 201 ± 30 h). Le déplacement des smolts dans l'estuaire révélait un schéma migratoire sélectif favorisant le transport nocturne par la marée descendante. Tous les smolts descendaient vers la mer avec le flot de jusant, près de la surface, et dans la section de colonne d'eau qui se déplaçait le plus vite. Le schéma nocturne de migration semble être le résultat d'un rythme endogène d'activité de nage qui pousse les smolts montrer dans la colonne d'eau après le crépuscule et à migrer vers la mer. La migration des smolts dans le bas estuaire indiquait une nage dirigée active, et il ne semblait pas y avoir de période d'accoutumance pour le passage de l'eau douce à l'eau salée.

[Traduit par la Rédaction]

## Introduction

In recent years there has been increasing concern about the potential impact of estuarine developments such as power stations and barrages on the behaviour and survival of migratory salmonids. Of particular concern are the effects on the seaward migration of Atlantic salmon (*Salmo salar* L.) smolts. The migration of smolts from fresh water to the marine environment is considered to be a particularly critical period for the survival of salmon (Doubleday et al. 1979; Browne et al. 1982). The timing of this movement has

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been suggested to be crucial to the survival and return as adult fish (Larsson 1977; Cross and Piggins 1982; Hansen and Jonsson 1989). An understanding of the environmental and physiological mechanisms controlling the behaviour and movement of Atlantic salmon smolts through estuaries is therefore important for stock management.

Most previous telemetry studies on the estuarine behaviour of Atlantic salmon smolts have utilized hatchery-reared fish (Fried et al. 1978; McCleave 1978; Tytler et al. 1978; Greenstreet 1992*a*). However, it is not known whether the migratory behaviour of hatchery-reared smolts can be compared with that of wild fish. More recently, studies on the behaviour of wild smolts through the estuary of the River Avon, southern England, have been successfully undertaken with the use of a miniature acoustic transmitter (Moore et al. 1990*a*, 1992). Movement through this relatively short estuary was nocturnal, within a single tidal cycle, and occurred predominantly on an ebbing tide. To increase our understanding of the mechanisms controlling the migratory behaviour of smolts within estuaries, a further study was undertaken in a more extensive system where conditions could result in longer residency.

In 1992 and 1993, the behaviour of wild Atlantic salmon smolts was studied during migration from fresh water to the marine environment through the River Conwy estuary, North Wales. Migrating wild smolts were trapped and tagged with miniature acoustic transmitters in the freshwater section of the river. This paper describes the behaviour of the smolts during the migration and discusses the results in relation to the environmental and physiological cues initiating and controlling estuarine migratory behaviour.

## Methods

### Study site

The River Conwy rises from the Lyn Conwy in the Migneint Moor in Snowdonia, Wales, drains a catchment of 590 km$^2$, and discharges to Liverpool Bay at Conwy after flowing 56 km. The Conwy estuary is a typical drowned river valley created by the extensive flooding of lowland areas during the general rise in sea level that occurred at the end of the Pleistocene epoch. Physical conditions have been extensively studied within the estuary (Knight and West 1975; Hunter and Lailey 1980; Pelegri 1988) and the following physical description is based upon both these accounts and data collected during the present study. Mean tidal amplitudes are 3.8 m on neaps and 6.0 m on springs, values that are significantly lower than those on the open coast because of a narrowing of the channel at Deganwy (Fig. 1). Nevertheless, current speeds are considerable, with maximum values of 2.71 $m \cdot s^{-1}$ (upper estuary) in a seaward direction recorded on the ebb tide during the study, and 2.59 $m \cdot s^{-1}$ (middle estuary) in a landward direction on the flood. These currents result in sufficient mixing to minimize stratification on both ebb and flood tides.

Horizontal current velocity profiles of the estuary during an ebb and a flood tide indicated that current speeds were significantly greater in the upper water column than the lower water column throughout the middle and lower estuary. Typical current speeds in the middle estuary, measured 2 h 40 min after high water, were 1.52 $m \cdot s^{-1}$ in the upper 30% of the water column and 0.69 $m \cdot s^{-1}$ in the lower 30% of the water column. Maximum current speeds also occurred in the vicinity of the main channel.

Despite the differences in speed between spring and neap tides, there is mixing of fresh and salt water on both, with maximum current speeds in the middle estuary ranging from 1.55 $m \cdot s^{-1}$ on springs to 0.94 $m \cdot s^{-1}$ on neaps. Some stratification occurs in the lower estuary between 2.5 and 3 h after low water, but this is soon broken down by the flood tide. Consequently little vertical or lateral stratification (<2 practical salinity units (psu); Unesco 1985) occurs elsewhere in the estuary. As the duration of the flooding tide decreases upstream, there is a longer period of river flow at low water. At low tide the estuary drains and consists of a channel of fresh or brackish water almost to the mouth, where stratification occurs.

During the period of the study in 1992, the minimum and maximum river flows recorded at the river gauging station at Cwm Llanerch (53°06′N, 3°47′W) were 5 and 113 $m^3 \cdot s^{-1}$, respectively (average daily flow 17.4 $m^3 \cdot s^{-1}$). In 1993, the minimum and maximum river flows recorded at Cwm Llanerch were 1.9 and 193 $m^3 \cdot s^{-1}$, respectively (average daily flow 12.9 $m^3 \cdot s^{-1}$).

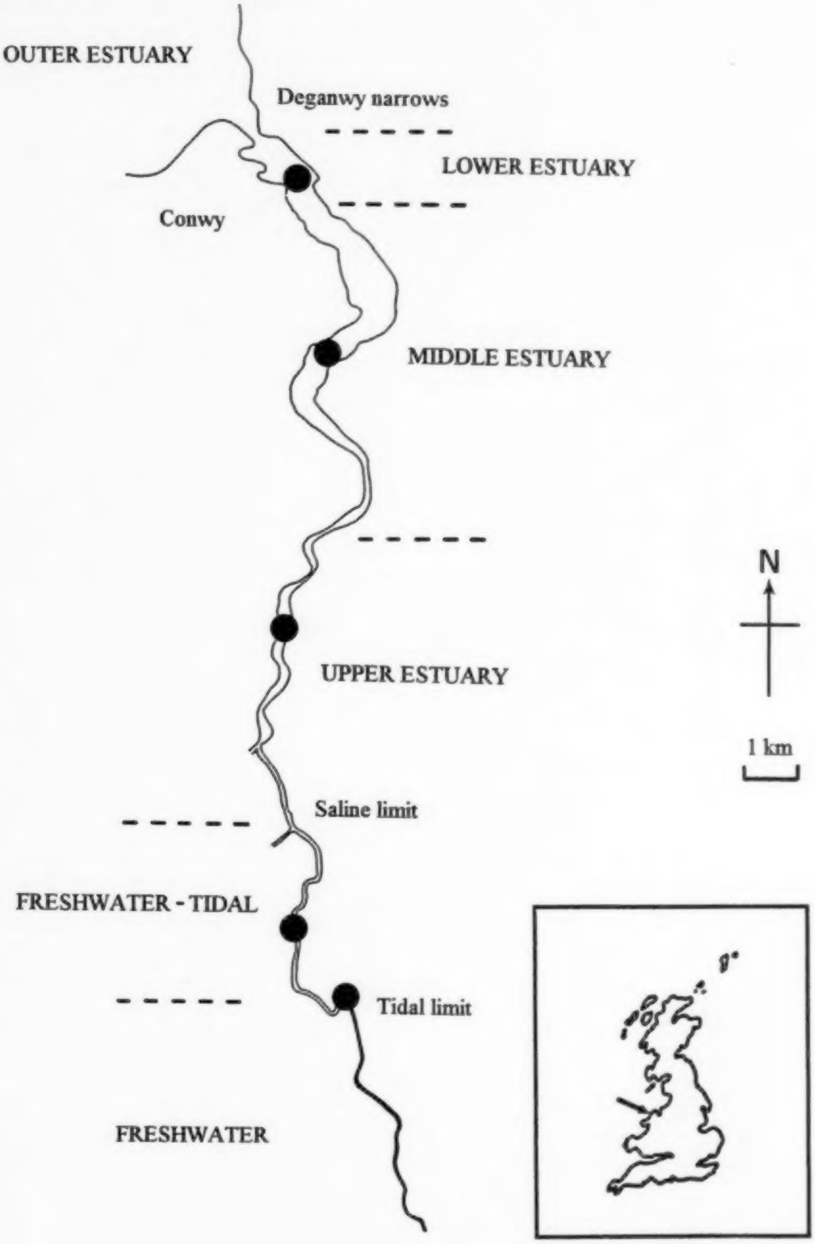
### Smolt trapping

The study was carried out over 2 years, between 29 March and 5 May 1992 and between 13 April and 22 May 1993. During 1992, migrating Atlantic salmon smolts were trapped at two sites. The first was on the River Conwy (53°06′N, 3°47′W), 6.4 km above the head of tide, and the second was on a small tributary of the main river, the Nant-y-goron (53°07′N, 3°47′W), 3.3 km above the head of tide. During 1993, smolts were only trapped on the main river. Migrating smolts were trapped using standard fyke nets attached to a 0.5 × 0.5 × 0.5 m keep box. In both years the traps were operated during dusk and the hours of darkness starting at approximately 18:00, and operation was continued until sufficient smolts had been caught and released. This usually occurred by 02:00 the following day. The traps were also operated on both the main river and the Nant-y-goron for periods of 24 h continuously to investigate the daily pattern of smolt behaviour. In 1992 these 24-h trapping studies were carried out on 7–8 April on the Nant-y-goron and 9–10 and 23–24 April on the main river. In 1993, the trap was operated on the main river for a 24-h period between 28 and 29 April.

### Transmitter attachment

Smolts were tagged using miniature 300-kHz acoustic transmitters, which had been developed at the Ministry of Agriculture, Fisheries and Food (MAFF) Fisheries Laboratory, Lowestoft, U.K. (Moore et al. 1990*b*). The transmitter circuitry and battery were enclosed in a cylindrical polycarbonate case with hemispherical ends. The tags were 17 mm long × 8 mm in diameter, and weighed 1.30 g in air and 0.35 g in water. The transmitter had a maximum range in the estuarine environment of around 100 m, although under most operating conditions the range will be 50–75 m because of varying hydrographic conditions such as turbidity, air entrainment, salinity, current speed, and suspended solids. Transmitting at 60 pulses/min, the tags had

**Fig. 1.** Map of the River Conwy showing the five sections of the river referred to in the text. The positions of the acoustic signal relay buoys in each section used to calculate the movements of smolts with respect to mean time of day and mean time in relation to the tidal cycle are denoted by ●.


OUTER ESTUARY
Deganwy narrows
LOWER ESTUARY
Conwy
MIDDLE ESTUARY
N
UPPER ESTUARY
1 km
Saline limit
FRESHWATER - TIDAL
Tidal limit
FRESHWATER

an operating life of approximately 30 d. Individual fish were identified by using tags with different pulse rates (range 40–100 pulses/min).

The transmitters were surgically implanted into the peritoneal cavity of the smolts. This technique of transmitter attachment has been shown to have negligible physiological and behavioural effects on salmon smolts (Moore et al. 1990*c*), and has previously been used successfully to track both salmon and sea trout (*Salmo trutta*) smolts within the River Avon (Moore et al. 1990*a*, 1992; Moore and Potter

1994). After transmitter attachment, the smolts were transferred to a tank of oxygenated water and allowed to recover fully from the effects of anaesthesia as described by Moore et al. (1990*c*). Recovery was usually within 5–10 min, and the fish were released 30–60 min after capture together with a number (three to five) of untagged smolts trapped at the same time.

### Acoustic tracking system

The movement of the smolts within the riverine and estuarine sections of the River Conwy were monitored using an array of 300-kHz acoustic signal relay buoys (Moore et al. 1992; Moore and Potter 1994). During the 1992 season, the buoys were positioned between the limit of tidal influence and the exit of the estuary (Fig. 1). In 1993, additional buoys were deployed to extend the coverage of the upper, middle, and lower estuary. Nine automatic listening stations were deployed along the length of the river and estuary to record signals from the acoustic buoys. Smolts within the riverine section of the River Conwy were also located at various times throughout the study. A hand-held 300-kHz acoustic hydrophone together with a Lowe HF-150 communications receiver (Lowe Electronics Ltd., Matlock, England) were used to locate smolts believed to be holding position within the river during daylight hours. Data on the river flow and river depth in the vicinity of the fish during this time were then obtained.

### Data analysis

For the purpose of this study the River Conwy was divided into five sections (Fig. 1) adopted from the description by Hunter and Lailey (1980) and based upon saline and tidal characteristics. These sections are (*i*) freshwater, trap site to tidal limit (4.7 km in length); (*ii*) freshwater–tidal, tidal limit to the maximum extent of saline influence (4.0 km in length); (*iii*) upper estuary (4.15 km in length); (*iv*) middle estuary (6.5 km in length); and (*v*) lower estuary (2.0 km in length).

The mean time of day and the mean time in the tidal cycle at which the smolts were detected by the acoustic signal relay buoy closest to the middle of each section (Fig. 1) were calculated using vector analysis (Batschelet 1981). The lengths of the mean vectors were further used to test whether movement of the smolts through each section was random with respect to time and state of tide using the Rayleigh test (*r* value) (Batschelet 1981). High water at Conwy was used as a reference time for all tidal cycles. Movement past each acoustic buoy in relation to tidal cycle was calculated using this reference time together with the appropriate phase lag at each point within the estuary derived from Hunter and Lailey (1980).

The relationships between the total time taken for the fish to leave the estuary and the day of release as well as mean river flow were examined using regression analysis and the line was fitted using the least-squares method.

### Environmental data collection

Environmental monitoring equipment was placed at a number of sites within the riverine and estuarine sections of the River Conwy. In 1992, three RCM 7 recording current meters (Aanderaa Instruments, Bergen, Norway) collected data on water velocity, temperature, and salinity at three fixed sites within the middle and lower estuary. During the same period four Datasonde 3 multiparameter water quality data loggers (Hydrolab, Luton, England) were placed at the tidal limit, at the saline limit, and in the middle and lower estuary. These provided data relating to depth, salinity, pH, dissolved oxygen content, and temperature. In 1993, two RCM 7 recording current meters were positioned for varying periods of time adjacent to acoustic buoys throughout the estuary and three Datasonde loggers were placed in the freshwater–tidal section and middle and lower estuary. In 1992 and 1993, vertical salinity profiles of the estuary were obtained at a number of sites using a Mini STD SD202 probe (Sensordata, Bergen, Norway).

The times taken for passively drifting objects (oranges) to travel the length of the upper, middle, and lower estuary sections were also measured. Batches of three oranges were released in the upper and middle estuary sections at the same time in the tidal cycle that smolts had been detected at adjacent acoustic buoys. Where individual oranges drifted out of the main flow or washed ashore, additional oranges were released within the main flow. This allowed us to determine the time for an inert object, remaining within the main flow, to traverse the length of the estuary.

In 1992 and 1993, current velocity profiles of the estuary were obtained using a 600-kHz broad band acoustic doppler current profiler (ADCP; RD Instruments, San Diego, Calif.). In 1992, the ADCP, towed behind a small research vessel, obtained transverse water velocity profiles and in 1993 it obtained longitudinal profiles of the middle and lower estuary sections. Profiles were obtained during both neap and spring tidal cycles. The current profiles obtained have been related to what is known of the smolts spatial and temporal positions within the middle and lower estuary sections in an effort to better understand their migration with respect to flow.

### Swimming behaviour in Atlantic salmon smolts

A preliminary laboratory study was carried out to investigate swimming behaviour of smolts. Six smolts were trapped on the Nant-y-goron between 20 and 21 April 1992 and transported in water- and oxygen-filled polythene bags to the School of Ocean Sciences, Menai Bridge. Rhythmic behaviour was studied in a purpose-built actograph, 0.9 m in length × 0.3 m in depth × 0.3 m in width. The flow rate through the actograph was 2 $\mathrm{L \cdot min^{-1}}$ and the temperature was 10.5 ± 0.5°C (mean ± SD).

Six infrared beams were located at depths of 0.1 and 0.185 m across the width of the actograph. These enabled the vertical patterns of movement within the water column to be described. The number of beam breaks at each depth as a result of movement by the smolts was totalled and logged on computer at 10-min intervals.

Two experiments were conducted. In the first, swimming activity in fresh water was studied. Three smolts were placed together in the actograph and allowed to acclimatize for 7 h. Activity was then examined over a 108-h period with the light regime D:L:D:L:$\underline{\mathrm{L}}$:L:D:$\underline{\mathrm{D}}$:D, each period lasting 12 h. The underlined conditions disrupted the expected pattern of light and dark. In the second experiment, swimming activity in salt water was studied. Three smolts were initially placed together in a large recirculating tank of fresh water

**Table 1.** Summary of information relating to Atlantic salmon smolts trapped in the River Conway in 1992 and 1993.

| Year | Date | Capture time | Length (mm) | River temperature (°C) | Time of capture after nautical twilight | Capture and release site | Residency in River Conwy (h) |
|---|---|---|---|---|---|---|---|
| 1992 | 09 Apr. | 22:40 | 158 | 9.4 | 2 h 28 min | Nant-y-goron | 106.2 |
| | 10 Apr. | 00:40 | 168 | 9.4 | 4 h 28 min | Nant-y-goron | 315.2 |
| | 14 Apr. | 21:05 | 158 | 8.1 | 0 h 45 min | Nant-y-goron | 267.1 |
| | 14 Apr. | 20:45 | 164 | 8.1 | 0 h 25 min | Nant-y-goron | 301 |
| | 14 Apr. | 21:30 | 155 | 8.1 | 1 h 20 min | Nant-y-goron | 296.5 |
| | 18 Apr. | 22:30 | 163 | 10.2 | 1 h 57 min | R. Conwy | 327.2 |
| | 18 Apr. | 22:30 | 153 | 10.2 | 1 h 57 min | R. Conwy | 199 |
| | 25 Apr. | 22:24 | 164 | 11.1 | 1 h 37 min | R. Conwy | 135 |
| | 25 Apr. | 22:35 | 173 | 11.1 | 1 h 46 min | R. Conwy | — |
| 1993 | 20 Apr. | 22:55 | 155 | 10.4 | 2 h 21 min | R. Conwy | 234.7 |
| | 21 Apr. | 00:01 | 138 | 10.4 | 3 h 27 min | R. Conwy | 576 |
| | 21 Apr. | 00:01 | 142 | 10.4 | 3 h 27 min | R. Conwy | 535.2 |
| | 21 Apr. | 00:10 | 144 | 10.4 | 3 h 36 min | R. Conwy | 376.5 |
| | 22 Apr. | 22:15 | 149 | 9.9 | 1 h 43 min | R. Conwy | 351 |
| | 27 Apr. | 23:00 | 150 | 11.1 | 2 h 11 min | R. Conwy | 282.5 |
| | 27 Apr. | 24:00 | 163 | 11.1 | 3 h 11 min | R. Conwy | 432.2 |
| | 27 Apr. | 24:00 | 171 | 11.1 | 3 h 11 min | R. Conwy | 367 |
| | 27 Apr. | 24:00 | 151 | 11.1 | 0 h 42 min | R. Conwy | 253 |
| | 02 May | 21:45 | 163 | 10.9 | 1 h 37 min | R. Conwy | 448.25 |
| | 02 May | 22:40 | 135 | 10.9 | 2 h 27 min | R. Conwy | 286 |
| | 02 May | 23:30 | 156 | 10.9 | 2 h 27 min | R. Conwy | 340.3 |
| | 02 May | 23:30 | 147 | 13.6 | 0 h 42 min | R. Conwy | 312.5 |
| | 07 May | 22:00 | 145 | 13.6 | 0 h 42 min | R. Conwy | 155.5 |
| | 07 May | 22:00 | 154 | 13.6 | 2 h 12 min | R. Conwy | 294 |
| | 07 May | 23:30 | 154 | 12.4 | 2 h 12 min | R. Conwy | — |
| | 09 May | 22:50 | 137 | 12.4 | 2 h 12 min | R. Conwy | 79 |
| | 09 May | 23:30 | 181 | 12.4 | 2 h 12 min | R. Conwy | 79 |
| | 09 May | 23:30 | 157 | 12.4 | 2 h 12 min | R. Conwy | 106 |
| | 09 May | 23:30 | 151 | 12.4 | 2 h 12 min | R. Conwy | 167 |
| | 09 May | 23:30 | 150 | 12.4 | 2 h 12 min | R. Conwy | 80 |
| | 09 May | 23:30 | 153 | 12.4 | 2 h 12 min | R. Conwy | 191.2 |
| | 09 May | 23:30 | 140 | 12.4 | 2 h 12 min | R. Conwy | 145.7 |
| | 09 May | 23:30 | 137 | 12.4 | 2 h 12 min | R. Conwy | 145 |

(capacity 800 L; temperature 10.0 ± 0.8°C) and maintained in a 12 h L : 12 h D light regime. Salinity was then increased from 0 to 32 psu over 3 days using clean seawater. The three smolts were then transferred together to the actograph containing clean seawater (32 psu), and allowed to acclimatize for 7 h. During the period of acclimatization, the smolts were maintained in a 12 h L : 12 h D light regime. Activity was then examined over a 108-h period with the light regime D:L:D:L:L:L:D:D:D, each period lasting 12 h. The underlined conditions disrupted the expected pattern of light and dark.

The mean number of beam breaks in the upper water column for each consecutive 12-h period was then calculated and compared with that for the previous period by using a paired *t* test. This provided a simple comparison of the swimming activity in the upper water column between night and day during both the actual and expected patterns of light and dark.

## Results

### Smolt trapping and tracking

In the 2 years of tracking, 31 of the 33 smolts released were tracked all the way through the estuary. In 1992, nine smolts were tagged and released at the two trapping sites (Table 1). Subsequently, eight smolts were tracked the length of the River Conwy and were last detected by the acoustic buoy at Deganwy narrows. The ninth smolt was detected moving only as far as the freshwater–tidal section.

In 1993, 24 smolts were trapped and released in the main river (Table 1). Of these, 23 smolts migrated past the lowermost estuary acoustic buoy at Deganwy narrows and the other fish as far as the freshwater–tidal section. Prior to moving past the tidal limit, six smolts were detected, by manual tracking, holding position in pools within the freshwater section. The current speed in these pools was 0.08 ± 0.04 $m \cdot s^{-1}$ and the depth was 0.94 ± 0.23 m.

**Table 2.** The downstream movements of Atlantic salmon smolts through each of the five sections of the River Conwy in relation to time of day in 1992 and 1993.

| | Freshwater | Freshwater–tidal | Upper estuary | Middle estuary | Lower estuary |
|---|---|---|---|---|---|
| 1992 | | | | | |
| Mean time | 22 h 10 min | 21 h 36 min | Insufficient data | 23 h 40 min | Random movement |
| *r* | 0.802 | 0.811 | | 0.632 | 0.26 |
| *p* | <0.01 | <0.02 | | <0.017 | >0.05 |
| *n* | 9 | 8 | | 10 | 8 |
| 1993 | | | | | |
| Mean time | 23 h 24 min | Random movement | 20 h 0 min | 23 h 32 min | 1 h 32 min |
| *r* | 0.574 | 0.293 | 0.424 | 0.532 | 0.322 |
| *p* | <0.001 | >0.05 | <0.04 | <0.001 | <0.05 |
| *n* | 24 | 34 | 18 | 28 | 27 |

**Note**: The mean times at which smolts were recorded passing the sonar buoy positioned closest to the middle of each section were calculated from the mean vectors (Batschelet 1981). The *r* values provide a measure of the randomness of the movement with respect to time calculated using the Rayleigh test; *n* is the total number of recordings of fish movements in a downstream direction by the respective sonar buoys.

In 1992 and 1993, all smolts were trapped during the hours of darkness. The first smolts to appear during each trapping session were recorded between 25 min and 4 h 28 min after nautical twilight (Table 1). Nautical twilight is defined as "the time when the level of illumination is such that the horizon is in general not visible and it is too dark for observation with a marine sextant" (Nautical Almanac 1991). Smolts then continued to be caught throughout the hours of darkness until cessation of the trapping operation. No smolts were trapped during the daylight hours of the 24-h continuous trapping sessions (1992 mean daily flow 7.3 $m^3 \cdot s^{-1}$ (first session) and 26.0 $m^3 \cdot s^{-1}$ (second session); 1993 mean daily flow 8.7 $m^3 \cdot s^{-1}$). This suggests that the overall sampling of the smolts was not significantly biased. Smolts were captured in the main river when river water temperatures were 11.25 ± 1.08°C (Table 1). In 1992, smolts were trapped in the Nant-y-goron when water temperatures were as low as 8.1°C (Table 1). However, these trapping sessions also coincided with elevated water levels and high coloration in the tributary.

The downstream movements of each smolt were detected by all of the acoustic buoys in fresh water and by the majority of the buoys in the freshwater–tidal, upper, middle, and lower estuary sections. This indicates that the fish must have passed within 50–75 m (the detection range) of the acoustic buoys, and thus probably followed the course of the low-water channel in the middle and lower estuary. However, smolts that subsequently moved upstream through the middle estuary section with the next flooding tide were not generally recorded until they reached the upper estuary. These fish may not have been detected by the acoustic buoys in the middle estuary because of an increase in suspended solids or because they moved upstream away from the main channel.

## Diurnal patterns of migration

During 1992 the overall movement of tagged smolts within the freshwater section of the River Conwy was predominantly nocturnal (Table 2). The mean time of day that the smolts passed the tidal limit of the river was 22:10, and the movement was nonrandom with respect to time. This nocturnal pattern of movement in smolts continued within the freshwater–tidal section of the River Conwy, with a mean time of transit of 21:36. Movement in the middle estuary section was also nocturnal (mean time of transit 23:40), but migration through the lower estuary section occurred during both the day and night.

In 1993 the mean time of day that smolts passed the tidal limit of the river was 23:24 and the movement was again nonrandom with respect to time. However, smolt movement became random in the freshwater–tidal section and the smolts were detected moving downstream during both day and night. A nocturnal pattern of movement was reestablished in the upper estuary (mean time 20:00). This predominantly nocturnal pattern of movement continued through the middle estuary (mean time 22:32) and lower estuary (mean time 01:32) sections.

## Run timing of smolts

Despite the predominantly nocturnal pattern of smolt migration overall, in 1993, seasonal differences were observed between smolts tagged at the beginning and end of the study (Table 3). In the freshwater section of the river, early-run smolts (tagged 20–27 April) demonstrated a nocturnal pattern of movement (mean time 23:24), whilst late-run fish (tagged on 2–9 May) migrated downstream during both day and night. Both groups of smolts retained their respective patterns of movement in the freshwater–tidal section (early-run smolts; mean time 22:12), but in the upper estuary, early- and late-run fish migrated during both day and night, and movement was random with respect to time. In the middle estuary, early- and late-run smolts switched back to a nocturnal pattern of movement (mean time: early-run, 00:36; late run, 22:40) and this was continued by late-run fish in the lower estuary (mean time 01:44), but not by early-run fish, which moved seaward during both day and night.

As the smolt season progressed, the released smolts spent less time within the River Conwy before migrating into coastal waters (Fig. 2; $r = -0.625$, $p < 0.00001$). The

**Table 3.** The downstream movements of early- and late-run Atlantic salmon smolts through each section of the River Conwy in relation to time of day in 1993.

| | Freshwater | Freshwater–tidal | Upper estuary | Middle estuary | Lower estuary |
|---|---|---|---|---|---|
| Early run | | | | | |
| Mean time | 23 h 24 min | 22 h 12 min | Random movement | 0 h 36 min | Random movement |
| *r* | 0.628 | 0.612 | 0.309 | 0.684 | 0.143 |
| *p* | <0.03 | <0.02 | >0.05 | <0.03 | >0.05 |
| *n* | 9 | 10 | 8 | 8 | 10 |
| Late run | | | | | |
| Mean time | Random movement | Random movement | Random movement | 22 h 40 min | 1 h 44 min |
| *r* | 0.189 | 0.368 | 0.213 | 0.572 | 0.578 |
| *p* | >0.05 | >0.05 | >0.05 | <0.003 | <0.02 |
| *n* | 16 | 15 | 15 | 18 | 12 |

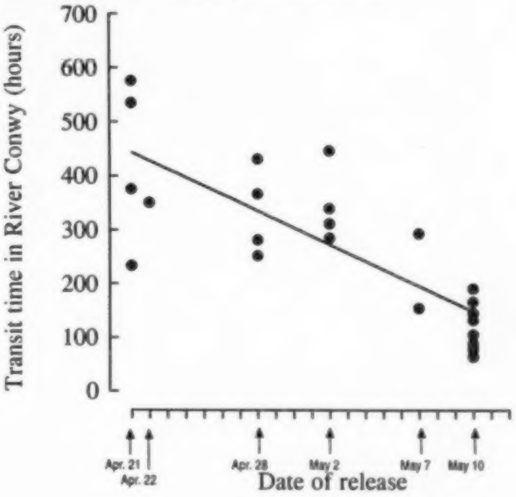
**Note:** The mean times at which smolts were recorded passing the sonar buoy positioned closest to the middle of each section were calculated from the mean vectors (Batschelet 1981). The *r* values provide a measure of the randomness of the movement with respect to time calculated using the Rayleigh test; *n* is the total number of recordings of fish movements.

mean time taken for smolts to migrate from the trap site to the exit of the estuary was not significantly different between the years 1992 and 1993 (Table 4; $p > 0.05$, two-tailed $t$ test assuming unequal variances). In 1992 the mean time spent by smolts in the middle estuary section was significantly greater than in 1993 ($p < 0.001$; two-tailed $t$ test assuming unequal variances). During this time, fish were located in the upper reaches of the middle estuary where the mean current speed measured at 1 m depth was $0.26 \pm 0.23\ \mathrm{m \cdot s^{-1}}$. In 1993 the mean length of time that smolts remained within the middle estuary was less than two tidal cycles. In both years the mean transit time of smolts through the lower estuary was short and movement through this section was usually within a single tidal cycle.

A significant proportion of migration out of the estuary and into coastal waters was concentrated in a 5-day period that coincided with the last neap tide of the study (Fig. 3). As a result the number of tagged fish available to migrate increased until this time and then fell rapidly (Fig. 3). Although there was always a significant proportion of released fish within the river available to migrate out of the estuary, the majority of movement only occurred during this short period (Fig. 3). During this period there was no marked variation in either water temperature or flow.

There were insufficient data to carry out a similar analysis on the smolts tagged in 1992 and these data cannot be combined with the 1993 figures because of different effects of river conditions on migration in the 2 years.

**Fig. 2.** The relationship between transit time within all sections of the River Conwy after tagging and day of release of Atlantic salmon smolts in 1993. Details of the fitted regression line are given in the text.



### Tidal patterns of migration

Movements within the tidal sections of the River Conwy were significantly related to the direction of current flow and therefore the tidal cycle. In 1992 and 1993, downstream movement in the tidal sections occurred during an ebbing tide with mean times of between 2 h 37 min and 5 h 25 min, respectively, after high water (Table 5). Upstream movement within the tidal river was also significantly related to the direction of flow and the tidal cycle and only occurred during flood tides. Mean times after high water for upstream movement of smolts within the lower, middle, and upper estuary sections (1992 and 1993 data combined) were 9 h 01 min, 9 h 51 min, and 11 h 06 min, respectively (Table 6).

### Rhythmic movement within the estuary

Two distinct patterns of smolt movement were recorded in the middle and lower estuary sections of the River Conwy. The first was the movement of fish from the top of the middle estuary section and out into coastal waters within a single ebb tide. The second pattern was of fish remaining within the estuary for a number of tidal cycles and being transported in both directions with the prevailing tidal flow before finally moving out into coastal waters. The pattern of movement demonstrated by a smolt was dependent upon the time within the tidal cycle that it commenced the seaward

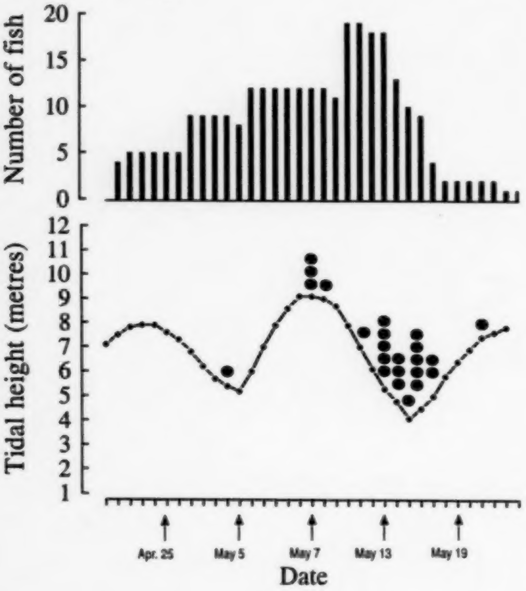
**Table 4.** Transit times of Atlantic salmon smolts in each of the five sections of the River Conwy in 1992 and 1993.

| River or estuary section | 1992 | 1993 |
|---|---|---|
| Freshwater | 100 h 26 min ± 153 h 30 min | 124 h 54 min ± 125 h 57 min |
| Freshwater–tidal | 64 h 25 min ± 88 h 50 min[a] | 67 h 26 min ± 71 h 12 min |
| Upper estuary | 64 h 25 min ± 88 h 50 min[a] | 30 h 45 min ± 31 h 10 min |
| Middle estuary | 112 h 40 min ± 151 h 35 min | 22 h 53 min ± 28 h 24 min |
| Lower estuary | 9 h 30 min ± 15 h 40 min | 4 h 26 min ± 3 h 27 min |

**Note**: Values are given as the mean ± SD.

[a] The data for the freshwater–tidal and upper estuary sections were combined for 1992.

**Fig. 3.** The migration of Atlantic salmon smolts (●) out of the River Conwy estuary in relation to the spring–neap tidal cycle in 1993. The tidal height is the difference between the high and low waters each day. The histogram shows the numbers of tagged fish available to emigrate through the estuary.



migration from the top of the middle estuary section (Figs. 4*a* and 4*b*). Smolts that were successful in moving through the estuary on a single ebb tide moved seaward during the early phase of the tide (mean time 1 h 39 min after high water; $r = 0.886$; $p < 0.001$; Fig. 4*a*). Smolts that were unsuccessful and remained in the estuary throughout a number of tidal cycles commenced their seaward movements in the later half of the ebb tide (mean time 5 h 01 min after high water; $r = 0.834$; $p < 0.001$; Fig. 4*b*). These fish normally encountered a flood tide before they reached the exit of the estuary, and were passively transported back upstream to the top of the middle estuary section. They then maintained position close to a road bridge in this section of the estuary where the current speed, measured at 1 m depth, was $0.26 \pm 0.23\ \mathrm{m \cdot s^{-1}}$.

Smolts initially unsuccessful in migrating into coastal waters usually selected the next ebb tide during the hours of darkness (mean time of passing top of middle section, 22:16) to migrate seawards (Fig. 5). Movement was therefore nocturnal, which may indicate a selective nocturnal tidal pattern of migration through the middle and lower estuary.

### Active versus passive movement of smolts

The movement over the ground of smolts through the middle estuary section was not significantly different from that of an orange travelling within the main flow at comparable states of the tide ($t = 1.194$, df = 16, $p > 0.05$; two-tailed *t* test, unequal variances). However, within the lower estuary section smolts moved significantly faster over the ground than inert objects ($t = 2.49$, df = 16, $p < 0.01$; two-tailed *t* test, unequal variances). In addition, two smolts were recorded moving seaward through the lower estuary section during a flooding tide against the direction of flow. The measured flow rates during this period were 0.115 and $0.293\ \mathrm{m \cdot s^{-1}}$. These results suggest that smolts moved passively through the middle estuary section with the flow but with some degree of orientation that maintained them in the main current. However, on reaching the lower estuary section, they may begin to move at speeds in excess of the current's.

Smolts migrating through the upper and middle estuary moved significantly faster during the hours of darkness than during daylight hours at comparable states of the tide (Table 7). This may suggest that, during the day, fish were attempting to maintain their position within the upper and middle estuary but were nevertheless being moved with the prevailing flow. However, within the lower estuary there were no significant differences in the speed between smolts moving during the day and during the night. This suggests that, on reaching the lower estuary section, fish no longer attempted to hold position during the day.

### Swimming behaviour of smolts: laboratory study

In fresh water, smolts demonstrated a nocturnal pattern of swimming activity. There was a significant increase in swimming activity in the upper water column during the hours of darkness ($t = 3.48$–$9.25$, df = 74, $p < 0.01$) (Fig. 6*a*). Manipulation of the light and dark pattern in periods 5 and 8 indicated that the fish also increased their activity in the upper water column during the times of expected darkness and decreased their activity during the

**Table 5.** The downstream movements of Atlantic salmon smolts through the tidal sections of the River Conwy in relation to the tidal cycle in 1992 and 1993.

| | Upper estuary | Middle estuary | Lower estuary |
|---|---|---|---|
| 1992 | | | |
| Mean time | Insufficient data | 3 h 24 min | 5 h 25 min |
| $r$ | | 0.882 | 0.702 |
| $p$ | | <0.001 | <0.02 |
| $n$ | | 10 | 8 |
| 1993 | | | |
| Mean time | 2 h 37 min | 3 h 57 min | 4 h 28 min |
| $r$ | 0.807 | 0.699 | 0.625 |
| $p$ | <0.001 | <0.001 | <0.001 |
| $n$ | 18 | 28 | 22 |

**Note**: The mean times are the times after high water that smolts were recorded passing the sonar buoy positioned closest to the middle of each section. Mean times were calculated from the mean vectors (Batschelet 1981). The $r$ values provide a measure of the randomness of the movement with respect to time calculated using the Rayleigh test; $n$ is the total number of recordings of fish movements in a downstream direction by the respective sonar buoys. Insufficient data were available to provide times for the freshwater–tidal section.

times of expected light. The pattern of activity of smolts in seawater was less clear than that recorded in fresh water (Fig. 6*b*). However, there was a peak of activity in the upper water column during the first 24 h after which the smolts again demonstrated a significant nocturnal pattern of swimming activity ($t = 3.22–4.13$, df = 74, $p < 0.01$) (Fig. 6*b*). Manipulation of the light and dark pattern in periods 5 and 8 resulted in activity very similar to that seen in the freshwater study.

### Environmental data

The greatest range in temperature, pH, salinity, and dissolved oxygen content was experienced in the upper and middle estuary. In the upper estuary, movement of smolts coincided with water temperatures of between 7.1 and 15.1°C. In the middle estuary, smolts experienced a range of pH values between 6.2 and 8.7 and salinities from 0.5 to 31.5 psu. The range of dissolved oxygen content throughout the estuary when fish were migrating was 6.1–11.2 $\mathrm{mg \cdot L^{-1}}$ with the lowest values being recorded in the upper estuary.

There was no apparent relationship between the observed changes in the pattern or rate of smolt movement through the estuary and the measured changes in water temperature or salinity. There was no evidence that movements were affected by changes in dissolved oxygen content although observed levels would not be expected to have any deleterious effects.

In 1992 and 1993 there was no relationship between the time each fish remained in the River Conwy after tagging and the mean river flow calculated during that time (Figs. 7*a* and 7*b*; 1992: $r = -0.006$, $p > 0.85$; 1993: $r = -0.007$, $p > 0.69$).

## Discussion

Thirty-one Atlantic salmon smolts were tracked the length of the River Conwy. Although this is only a small component of the total smolt run, the consistency in the spatial and temporal pattern of migration suggests that the results may be representative of the migratory behaviour of smolts in the River Conwy.

**Table 6.** The upstream movements of Atlantic salmon smolts through the lower, middle, and upper estuary in relation to the tidal cycle.

| | Upper estuary | Middle estuary | Lower estuary |
|---|---|---|---|
| Mean time | 11 h 6 min | 9 h 51 min | 9 h 1 min |
| $r$ | 0.924 | 0.933 | 0.9 |
| $p$ | <0.001 | <0.001 | <0.001 |
| $n$ | 7 | 9 | 7 |

**Note**: The mean times after high water at which smolts were recorded passing the acoustic buoy positioned closest to the middle of each section were calculated from the mean vectors (Batschelet 1981). The $r$ values provide a measure of the randomness of the movement with respect to time calculated using the Rayleigh test; $n$ is the total number of recordings of fish movement. The 1992 and 1993 data were pooled.

### Freshwater migration

Migration in the freshwater and freshwater–tidal sections of the River Conwy was predominantly nocturnal, supporting the observations from previous studies (Thorpe and Morgan 1978; Thorpe et al. 1981; Lundqvist and Eriksson 1985; Hansen and Jonsson 1985; Greenstreet 1992*a*; Moore et al. 1992), although there was a seasonal change in this nocturnal component of the migration (Osterdahl 1969; Solomon 1978). Nocturnal migration early in the smolt run switched to movement during both day and night as the season progressed. This change in the migratory pattern of behaviour was reflected in a significant seasonal change in the transit time of the smolts through the estuary, with fish tagged later in the season spending less time in the River Conwy before migrating into coastal waters. As a consequence in 1993 a significant proportion of the tagged smolts migrated out of the estuary over a 5-day period. This period may represent the optimal time or window of

**Fig. 4.** The timing of seaward movements of Atlantic salmon smolts past the upper limit of the middle estuary section in relation to the tidal cycle. (*a*) Smolts that were successful and (*b*) smolts that were unsuccessful in moving through the middle and lower estuary sections and into coastal waters on a single ebb tide. The *r* value is the measure of variation provided by the Rayleigh test.

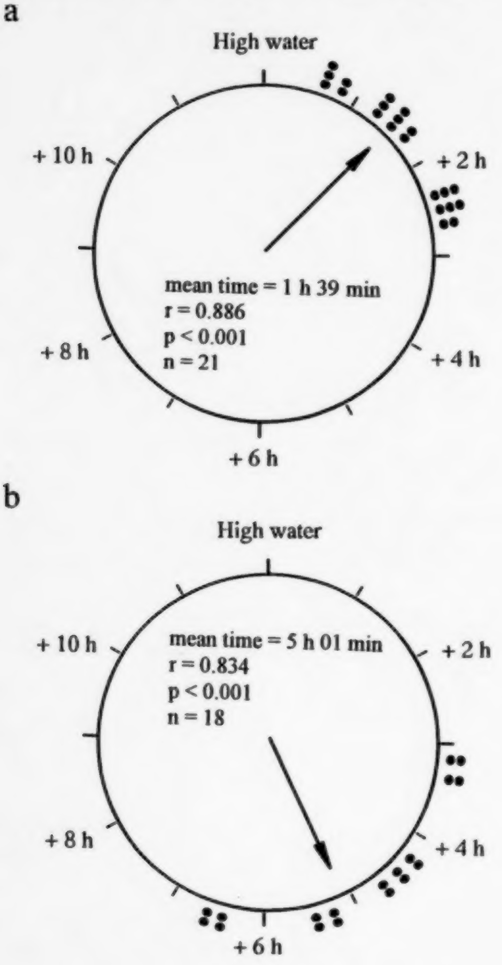


**Fig. 5.** The time of seaward movements of Atlantic salmon smolts past the upper limit of the middle estuary section after a previous unsuccessful attempt to move out of the estuary on a single ebb tide. The *r* value is the measure of variation provided by the Rayleigh test.

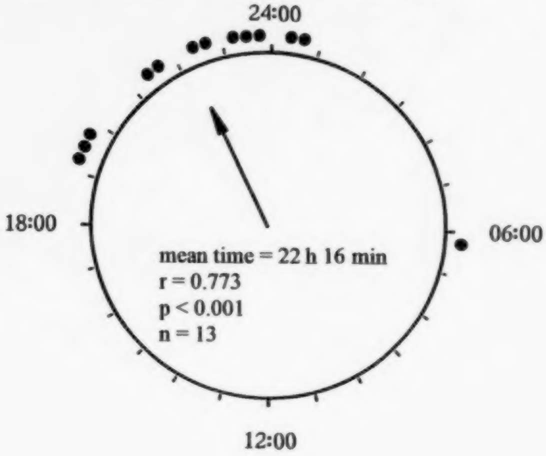


**Table 7.** The times taken for Atlantic salmon smolts to migrate through the upper, middle, and lower estuary during day and night in 1992 and 1993.

| | Upper estuary | Middle estuary | Lower estuary |
|---|---|---|---|
| Day (min) | 234.8 | 218.2 | 51.1 |
| Night (min) | 121.8 | 141.6 | 66.6 |
| df | 6 | 21 | 33 |
| *t* | 4.704 | 5.234 | 0.995 |
| *p* | <0.01 | <0.001 | ns |

**Note**: Data between day and night were compared using a *t* test assuming unequal variances.

opportunity for smolts to migrate from the River Conwy into the marine environment. It has previously been observed that the timing of the migration of Atlantic salmon smolts from fresh to salt water is critical to their subsequent survival and return as adults (Larsson 1977; Cross and Piggins 1982; Hansen and Jonsson 1989). However, it is not understood what environmental mechanisms are operating during this initial marine phase that make this timing so crucial.

The nocturnal pattern of migration would appear to be the result of an endogenous rhythm of swimming activity that results in fish moving into the upper water column after dusk and migrating downstream. This hypothesis is supported by the laboratory-based behaviour study, which suggests that the light–dark cycle may act as a zeitgeber. It is unlikely that the rhythm of swimming activity is controlled solely by changes in ambient light intensity as suggested in previous studies (Hansen and Jonsson 1985; Greenstreet 1992*a*). The nocturnal pattern of migration was not evident in later running smolts, suggesting a modification to the clock component controlling swimming behaviour. Departure in later run smolts from the predominantly nocturnal pattern of migration may be related to ensuring that they reach coastal waters at the optimal time.

Nocturnal migration may reduce the chances of avian predation particularly by cormorants (*Phalacrocorax carbo*) and herons (*Ardea cinerea*), which prey on smolts in the River Conwy (A. Moore, personal observation). Levels of predation by birds on Atlantic salmon smolts as high as 70% have been estimated on some rivers (Larsson 1985; Kennedy and Greer 1988). As the majority of piscivorous birds feed visually during the day (Kennedy and Greer

**Fig. 6.** Swimming activity of Atlantic salmon smolts in a 0.9 × 0.3 × 0.3 m tank containing (*a*) fresh water ($n = 3$) and (*b*) seawater ($n = 3$). The mean swimming activity in the upper water column for each subsequent 12-h period has been plotted. The hatched areas indicate where the expected pattern of light and dark periods have been disrupted. There were significant differences in activity in the upper water column between all subsequent 12-h periods ($p < 0.01$), except where indicated. Error bars represent SEM.

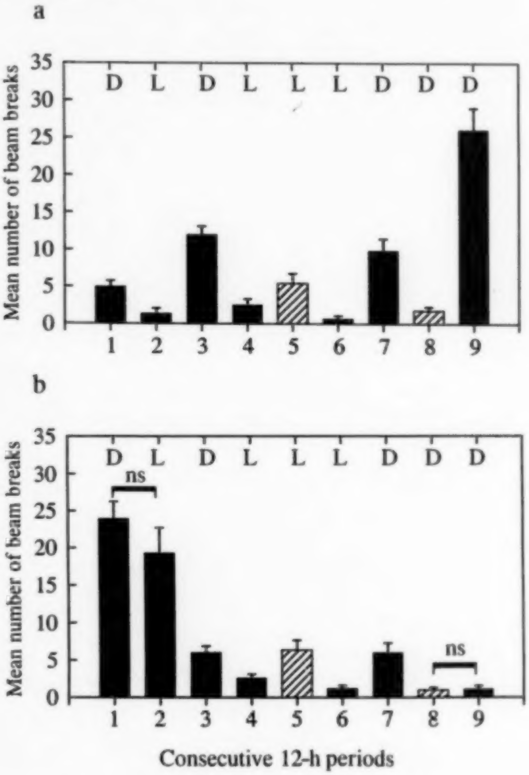
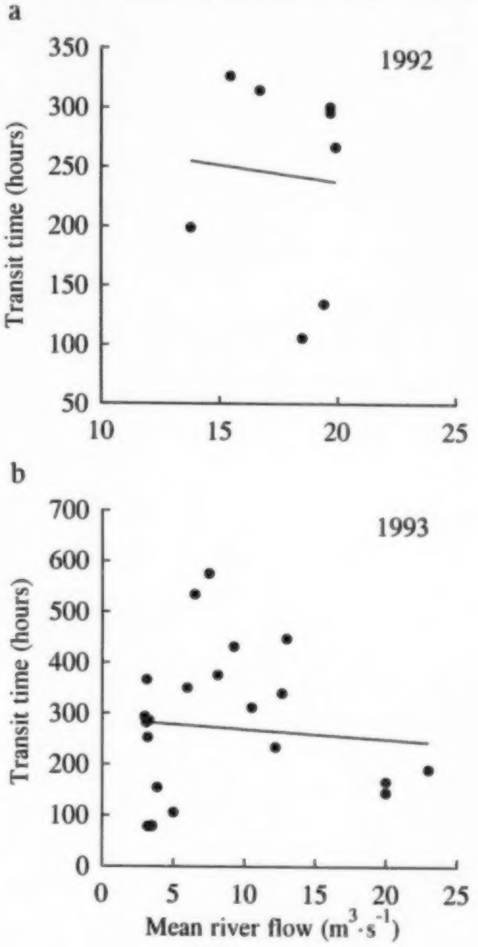


**Fig. 7.** The relationship between the transit time of tagged Atlantic salmon smolts between release and exit from the River Conwy estuary and the mean river flow during the period of transit in (*a*) 1992 and (*b*) 1993. Details of the fitted regression lines are given in the text.



1988), refuging close to the bed of the river during the day and migrating at night may reduce predation pressure.

A number of other environmental factors may also initiate and modify smolt migration in fresh water. Migration may occur when a certain temperature threshold has been exceeded (Osterdahl 1969; McCleave 1978) or be initiated by variations in the patterns of temperature over a prolonged period (Jonsson and Ruud-Hansen 1985). During this study, smolt migration in the main river coincided with temperatures of above 10°C, the threshold reported in other studies (Osterdahl 1969; Solomon 1978). Movement in the tributary of the River Conwy occurred at much lower temperatures but coincided with a sudden increase in flow rate, which has also been implicated in the initiation of smolt migration (Solomon 1978). The movement of smolts in the tributary may have been the result of flushing, whereby the fish were unable to maintain position and were physically transported into the main river. In the main river there was no observable correlation between increased water flow and smolt migration in any of the sections of the Conwy. However, it is possible that more subtle changes in water flow, not evident from the data recorded at the gauging station, may have had an effect on the movement of smolts.

### Estuarine migration

There were significant spatial and temporal differences in the patterns of migration of smolts in the estuary of the River Conwy. In the freshwater–tidal and upper sections of the estuary, there was a strong tidal component to the direction of migration. Movement was predominantly with the direction of flow (McCleave 1978) and a nocturnal pattern of migration was only evident in the smolts tagged early in the season.

In the middle estuary section, the results indicate the presence of a nocturnal selective ebb tide transport component to the smolt migration, similar to mechanisms demonstrated in other species (Harden Jones et al. 1979). Smolts demonstrated a strong nocturnal tidal pattern of migration, and movement seaward tended to occur on an ebb tide during the hours of darkness. Previous studies have also suggested that salmon smolts initiate their seaward movement through an estuary on an ebb tide (Calderwood 1908; White and Huntsman 1938). It is suggested that during the present study smolts migrated close to the surface (Fried et al. 1978; Holm et al. 1982) and oriented to remain within the main flow of the channel. In the lower estuary, the pattern of movement was predominantly the same, although during 1992, migration occurred on ebb tides during both the day and night. Smolts migrating on an ebb tide close to the surface and within the main channel of the middle and lower estuary are within the section of the water column with the highest current velocities. Energetically this is the most advantageous strategy for migration through the estuary and would result in the rapid movement of smolts out into coastal waters.

It has been argued that smolt migration in estuaries is passive (Huntsman 1962; Tytler et al. 1978; Thorpe et al. 1981; Greenstreet 1992*b*), partly active (Thorpe and Morgan 1978; McCleave 1978; Solomon 1978; Kennedy et al. 1984), or active (Hansen and Jonsson 1985; Moore et al. 1992). In the River Conwy, migration through the estuary varied both spatially and temporally. Migration through the upper and middle estuary was passive during the hours of darkness but with some degree of orientation that maintained the smolts in the upper water column and within the main current. During daylight hours, movement was significantly slower. This difference in migratory behaviour may be explained by fish attempting to refuge close to the bed of the estuary during daylight hours but nevertheless being unable to maintain station in the tidal currents. During periods of ebb flow, maximum current speeds in the upper and middle section of the estuary exceeded 15 body lengths/s, which is in excess of the flows at which smolts can maintain station (Wankowski and Thorpe 1979).

Smolt movement through the lower estuary was indicative of active, directed swimming. Migration was at speeds significantly in excess of the current and in two instances against the direction of flow. LaBar et al. (1978) reported active swimming against the current by salmon smolts in the lower sections of a river estuary. Active tracking of the fish during this period again indicated that fish were in the upper water column and close to the maximum current speeds. A similar pattern of behaviour was demonstrated by smolts in the laboratory when they were transferred to seawater. It is possible that during the transition from fresh to salt water there is significant change in the smolts' behaviour and they switch from moving passively with the current to swimming actively seawards.

Movement through the estuary and into coastal waters was unaffected by changes in water quality. Increasing salinity did not affect the behaviour of the smolts (Greenstreet 1992*b*; Moore et al. 1992), and there was no apparent requirement for a period of saltwater acclimation as previously suggested in other salmonid smolts (Moser et al. 1991). This implies that physiological adaptation to a saline environment had previously occurred in fresh water (Primett et al. 1988). A physiological requirement to move to a saline environment may be an important cue in initiating smolt migration in the River Conwy.

An understanding of the environmental cues initiating smolt migration through estuaries is important in evaluating the potential impact of estuarine constructions such as barrages on the behaviour and survival of migratory salmonids. One major concern relates to the potential changes in the flow regimes above a barrage and the effects on smolt migration. The removal of a significant tidal cycle in the impoundment of a barrage would result in a reduction of the ebb tide cues that the smolts appear to use during their seaward movement. As a consequence of such an event, fish may reside for longer periods in an impoundment with the potential for an increase in predation, or the timing of the movement into coastal water may be delayed and may not occur at the optimal time. As previously noted, it has been suggested that the timing of smolt movement into coastal waters may be crucial to the survival and return as adult fish (Larsson 1977; Cross and Piggins 1982; Hansen and Jonsson 1989).

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# Response of the Laflamme Lake watershed, Quebec, to reduced sulphate deposition (1981–1992)

**Suzanne Couture**

**Abstract**: To verify the effectiveness of Canadian and U.S. programs to reduce $SO_2$ emissions, Environment Canada operates an aquatic ecosystem monitoring program that includes the Laflamme Lake integrated watershed north of Quebec City. Wet atmospheric sulphate deposition to the Laflamme Lake watershed declined between 1981 and 1991, particularly during the latter half of this period. The nitrate concentration increased in precipitation between 1981 and 1991. For the lake water, this resulted principally in a slow reduction in alkalinity between June 1981 and May 1992. After 1986, reductions in lake sulphate concentration were offset principally by reductions in Ca and Mg concentrations. An increase in lake water nitrate concentration was observed after 1986. The gradual loss of lake alkalinity demonstrates that the acid inputs to the lake are still too high for chemical recovery to occur. The potential improvement in lake alkalinity related to reduced sulphate concentration may be lost because of an increasing nitrogen-based acidification.

**Résumé** : Pour vérifier l'efficacité des programmes canadien et américain de réduction des émissions de $SO_2$, Environnement Canada gère un programme de surveillance des écosystèmes aquatiques incluant l'étude du bassin versant intégré du lac Laflamme au nord de la ville de Québec. Au bassin versant du lac Laflamme, les dépôts atmosphériques humides de sulfates ont diminué entre 1981 et 1991, et plus particulièrement au cours de la deuxième moitié de cette période. Les teneurs en nitrates des précipitations étaient à la hausse entre 1981 et 1991. Pour le lac, ceci s'est traduit principalement par une lente diminution de l'alcalinité entre juin 1981 et mai 1992. Après 1986, les réductions des concentrations lacustres de sulfates étaient principalement compensées par des réductions des concentrations de Ca et Mg. Une augmentation des teneurs en nitrates dans le lac a été observée après 1986. La perte graduelle de l'alcalinité du lac démontre que les charges acides parvenant au lac sont encore trop importantes pour qu'une récupération chimique ait lieu. La récupération potentielle de l'alcalinité du lac suite à la réduction des teneurs en sulfates a pu être retardée par une acidification accrue par les nitrates.
[Traduit par la Rédaction]

## Introduction

Canadian and U.S. acid rain control programs focus on reducing sulphur dioxide ($SO_2$) emissions. The $SO_2$ reduction objective for eastern Canada (i.e., east of and including Manitoba) set at 2.3 million metric tons (t) per year was to be achieved by 1994 and maintained until 1999. By 1992, the Canadian emission reduction objective had been essentially met (Canada – United States Air Quality Committee 1994). Signs of an improvement in precipitation quality during the 1980s, chiefly reduced sulphate concentration or wet deposition, were reported in eastern Canada (Sirois 1993), and specifically for the province of Quebec (Boulet and Pinard 1991; Papineau and Haemmerli 1992). However, Sirois (1993) also noted that declines in the sulphate concentration of precipitation were rarely monotonic.

To confirm the effectiveness of $SO_2$ reduction programs, Environment Canada has conducted a research program at the Laflamme Lake watershed (LLW) since 1981. The program investigates temporal changes and processes involved in acidification and recovery of an aquatic ecosystem following changes in the atmospheric deposition of acid-forming substances. North American studies have shown that changes in precipitation quality could trigger diverse changes in lake chemistry (Dillon et al. 1986; Kelso and Jeffries 1988; Driscoll et al. 1989; Dupont 1992; Papineau and Haemmerli 1992; Bouchard 1994). A period of acidification was observed in LLW between 1981 and 1985 when sulphate deposition increased slightly; lake sulphate concentration increased from 3.6 to 4.5 mg/L and alkalinity decreased from 6.75 to 5.50 mg/L (Papineau and Haemmerli 1992). From 1985 to 1988, lake sulphate concentration declined slightly (to 4.3 mg/L) without any observed increase in alkalinity.

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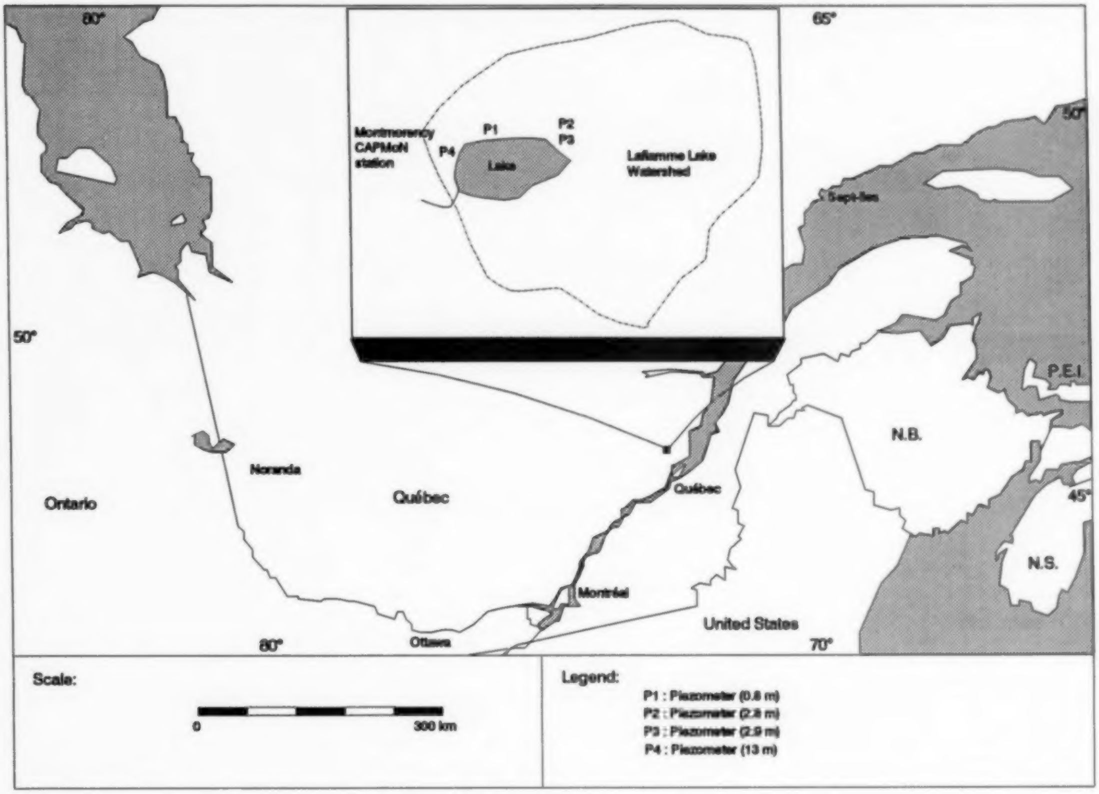
**Fig. 1.** Location of the Laflamme Lake watershed.



The purpose of this study is to present the water quality of three components (precipitation, surface water, and groundwater) of the LLW, the only boreal forest watershed study in the province of Quebec focusing on aquatic effects of acid deposition. The study focuses on recent patterns (especially the period after 1986) in atmospheric sulphate and nitrate depositions as well as the chemical response of pH, alkalinity, sulphate, nitrate, and Ca + Mg in lake water and groundwater. Finally, it examines whether a significant reduction in sulphate concentration has led to a concomitant recovery of lake alkalinity.

## Study area

The LLW is located 80 km north of Quebec City (47°19′N, 71°07′W, altitude 777–884 m) and covers 68.4 ha (Fig. 1). The lake itself has an area of 6.1 ha, an average depth of 2.05 m, and a maximum depth of 5.3 m and is frozen from mid November to mid May. This headwater lake is fed mainly by groundwater (Bernier et al. 1983) and water has a residence time of about 2 months in the lake. The bedrock of the watershed is made up of Precambrian charnockitic gneiss (Azzaria et al. 1982) and most of the surficial deposits are till and moraine composed predominantly of potassic feldspar, quartz, and plagioclase. The till can reach up to 15 m in depth under the lake yet varies only from 0.1 to 4 m in depth on the shores (Robitaille et al. 1983). The soils in the watershed are orthic ferro-humic podzols. The climate in the area is characterized by abundant precipitation and low temperatures: annual precipitation averages 1.32 m of which 33% is snow. The flow at the outlet stream represents 64% of precipitation to the watershed.

## Methods

### Precipitation

The Montmorency meteorological station near LLW is part of the Canadian Air and Precipitation Monitoring Network (CAPMoN) operated by Environment Canada (Fig. 1). Daily precipitation volume was obtained by standard Canadian meteorological service rain and snow gauges. For the chemical analyses, daily precipitation samples were collected using a wet precipitation sampler. The samples were maintained at 4°C without preservatives and shipped every 2 weeks to the National Laboratory for Environmental Testing in Burlington, Ontario. Measurements of pH were obtained using electrometric methods, while calcium and magnesium concentrations were analyzed by atomic absorption, and sulphate and nitrate concentrations by ion chromatography (Vet et al. 1989). Mean annual concentrations were calculated using volume-weighted

**Table 1.** Annual precipitation depth, volume-weighted mean concentration, and wet sulphate deposition for precipitation and annual means for lake-water concentrations at LLW from June 1981 to May 1992.

| | Precipitation | | | | | Lake | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $SO_4$ | | | | | | | |
| Year (June to May) | Depth (m) | pH | µequiv./L | kg/ha | $NO_3$ (µequiv./L) | pH | Gran alkalinity (µequiv./L) | Ca + Mg (µequiv./L) | $SO_4$ (µequiv./L) | $NO_3$ (µequiv./L) |
| 1981–1982 | 1.12 | 4.40 | 34.1 | 18.3 | 17.9 | 6.27 | 117.6 | 170.2 | 74.2 | 8.5 |
| 1982–1983 | 1.56 | 4.51 | 28.7 | 22.6 | 14.3 | 6.21 | 117.4 | 169.0 | 82.8 | 9.9 |
| 1983–1984 | 1.30 | 4.41 | 38.1 | 23.3 | 20.7 | 6.31 | 112.8 | 166.3 | 83.3 | 8.4 |
| 1984–1985 | 1.21 | 4.29 | 42.0 | 27.0 | 24.3 | 6.29 | 116.1 | 166.0 | 88.5 | 7.3 |
| 1985–1986 | 1.34 | 4.45 | 26.8 | 18.3 | 18.6 | 6.43 | 103.0 | 161.8 | 91.6 | 4.3 |
| 1986–1987 | 1.30 | 4.42 | 27.5 | 16.4 | 20.7 | 6.59 | 101.4 | 160.8 | 88.4 | 4.5 |
| 1987–1988 | 1.25 | 4.44 | 28.7 | 17.6 | 22.8 | 6.61 | 110.3 | 164.3 | 89.8 | 5.2 |
| 1988–1989 | 1.40 | 4.40 | 32.9 | 23.6 | 25.0 | 6.39 | 96.2 | 158.1 | 91.3 | 6.6 |
| 1989–1990 | 1.23 | 4.45 | 28.7 | 15.1 | 22.8 | 6.34 | 99.2 | 158.1 | 88.0 | 6.3 |
| 1990–1991 | 1.47 | 4.53 | 24.1 | 15.3 | 18.6 | 6.22 | 95.8 | 157.1 | 82.7 | 5.7 |
| 1991–1992 | 1.21 | 4.47 | 25.8 | 16.7 | 18.6 | 6.42 | 102.1 | 163.1 | 76.9 | 7.0 |
| Mean | 1.32 | 4.44 | 30.7 | 19.5 | 20.4 | 6.35 | 106.5 | 163.2 | 85.2 | 6.7 |

daily concentrations. Annual atmospheric inputs were obtained by summing the products of daily concentration and the daily precipitation amount.

### The lake

Integrated water samples taken at 0–4 m depth from the centre of the lake were collected every week from June 1981 to May 1992. Major ion analysis was carried out at the St. Lawrence Centre Laboratory (Montréal, Que.) using standard analytical methods outlined by Environment Canada (1979). The pH was measured using electrometric methods and alkalinity by Gran titration. Calcium and magnesium were analyzed using atomic absorption between 1981 and 1990, and subsequently by inductively coupled plasma techniques. Sulphate and nitrate were determined using a colorimetric autoanalyzer. Ion balance, calculated theoretical conductivity, as well as the relationship to historical concentration levels were all considered prior to acceptance of analytical results. Both the St. Lawrence Centre and Burlington laboratories participated in Long Range Transport of Airborne Pollutants interlaboratory studies to ensure quality control of measurements (Aspila and Chau 1990).

### Groundwater

Groundwater samples were collected beginning in 1988 on a bimonthly basis between May and November using four piezometers at depths of 0.8, 2.8, 2.9, and 13 m (Fig. 1). The three shallow piezometers were located in the recharge zone on the north shore, whereas the fourth one was in the discharge zone. The groundwater variables and analytical methods were the same as those described for the lake.

### Statistical analyses

The statistical analysis to identify trends was conducted using DETECT software (Cluis et al. 1988), which employs a multistep process designed to detect monotonic or step trends in water-quality variables using nonparametric methods. Except if specifically mentioned otherwise, all trends reported in this paper were significant at $\alpha = 0.05$. The software first evaluates seasonality in user-defined data groups (in this case, 3-month periods starting in June). Seasonality is accepted when the average of each season is significantly different using an analysis of variance (ANOVA). When significant seasonality is observed, the data used in the trend analysis are represented by an average value for each season. If no seasonality is identified, the data are represented by monthly averages.

The second step involves evaluating persistence of the data series using the autocorrelation coefficient. There is no persistence if the first-order autocorrelation coefficient is not significantly different from zero ($\alpha = 0.05$). The presence or lack of persistence in the data influences which statistical test is used to evaluate the trend.

The last step entails selecting and applying the appropriate nonparametric test to detect trends. The trend detection tests used in this study consisted of the seasonal Kendall test (minimum $N = 24$) in the case of series exhibiting seasonality (Hirsch et al. 1982), the Spearman–Lettenmaier test (minimum $N = 20$) for series showing persistence (Lettenmaier and Burges 1976), and the Hirsch and Slack test (minimum $N = 120$) for series with seasonality and persistence (Hirsch and Slack 1984). If a trend is detected in the time series, a regression line is calculated to determine the initial and final values of the series.

Since the groundwater data series included only 17 or 18 values, the Kendall test (minimum $N = 9$) was used for series that met the requirements for nonseasonality and nonpersistence (Hirsch et al. 1982). Series that did not have the characteristics required for the Kendall test were subjected to linear regression analysis. If the slope of the regression line for concentration versus time was significantly different from zero (two-tailed Student's $t$ test, $\alpha = 0.05$), an upward or downward trend, according to the sign of the slope, was declared.

**Table 2.** Trends in time series of precipitation and lake data at LLW.

| | Period | Trend | Persistence | Periodicity | *n* | Test |
|---|---|---|---|---|---|---|
| | | | **Precipitation** | | | |
| $SO_4$ concentration and deposition | 1981–1991 | Negative | No | Yes | 132 | Seasonal Kendall |
| $SO_4$ deposition | 1986–1991 | Negative | No | Yes | 72 | Seasonal Kendall |
| $NO_3$ concentration | 1981–1991 | Positive | No | Yes | 132 | Seasonal Kendall |
| | | | **Lake** | | | |
| Alkalinity | June 1981 – May 1992 | Negative | No | Yes | 571 | Seasonal Kendall |
| Ca + Mg concentration | June 1981 – May 1992 | Negative | No | Yes | 572 | Seasonal Kendall |
| $SO_4$ concentration | June 1981 – May 1986 | Positive | Markovian order = 1 | No | 258 | Spearman–Lettenmaier |
| $SO_4$ concentration | June 1986 – May 1992 | Negative | Markovian order = 1 | Yes | 312 | Hirsch and Slack |
| $NO_3$ concentration | June 1986 – May 1992 | Positive | No | Yes | 312 | Seasonal Kendall |

**Note**: For precipitation analyses, concentration and deposition series for $SO_4$, $NO_3$, $NH_4$, Ca, Mg, Ca + Mg, and pH (H) were tested ($\alpha$ = 0.05) for the complete (1981–1991) period and the period after 1985. For lake-water analyses, series for lake alkalinity, $SO_4$, $NO_3$, pH, and Ca + Mg concentrations were tested ($\alpha$ = 0.05) for the complete 1981–1991 period and the period after 1985.

## Results

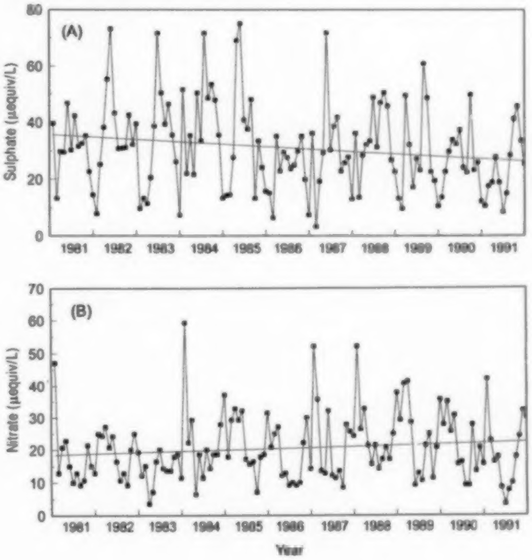
### Characterization of precipitation

The mean pH of precipitation for the 11-year period between June 1981 and May 1992 was 4.44 (Table 1). For the same period, the mean annual wet calcium, magnesium, sulphate, and nitrate deposition was 1.2, 0.23, 19.5, and 3.5 kg/ha, respectively. Both the interaction of precipitation with the forest canopy and the input by dry deposition affected the total amount of ions reaching the forest floor. According to Robitaille and Boutin (1990), calcium, magnesium, and sulphate inputs from incident precipitation are enriched 148, 382, and 15% respectively, when passing through the forest canopy (through-fall and stemflow water) in the LLW. Sirois and Barrie (1988) reported that dry deposition at the LLW meteorological station represented 14% of total annual inputs (dry + wet deposition) in the case of sulphate and 17% in the case of nitrate (excluding $NO_2$).

Between 1981 and 1991, significant declines in precipitation sulphate concentration and wet deposition were recorded (Table 2). The decrease in sulphate concentration at this site corroborates Sirois' (1993) results. The concentration dropped from 35.6 to 26.4 μequiv./L, a 26% decrease (Fig. 2A). Monthly atmospheric sulphate deposition also declined by 26%, from 1.9 to 1.4 kg/ha. From 1985 (the year the Eastern Canada Acid Rain Control Program was initiated) to 1991, monthly atmospheric sulphate deposition declined from 1.55 to 1.38 kg/ha, representing a decrease of 11% (Table 1). On an annual basis, this is about 2 kg/ha over the 6-year period.

Nitrate concentration rose from 18.6 to 22.1 μequiv./L between 1981 and 1991, an increase of 19% (Table 2, Fig. 2B). The upward trend in nitrate concentration was strongest (39%) between 1981 and 1989. After 1989, the slope of the trend decreased, but the positive slope for the overall period still remained significant. Precipitation pH did not show any significant change in response to the changes in sulphate or nitrate concentration. No trends were detected for calcium or magnesium concentrations, the sum of the calcium and magnesium concentrations (Ca + Mg), or the precipitation volume.

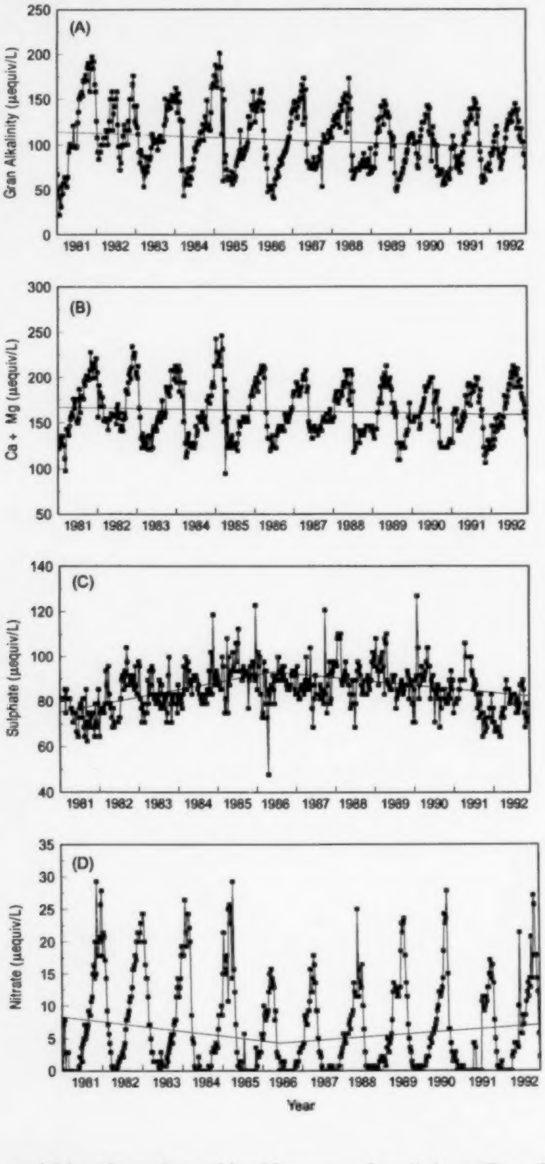
**Fig. 2.** Mean monthly precipitation concentration of sulphate (A) and nitrate (B) between 1981 and 1991.



### Characterization of lake water quality

Between June 1981 and May 1992, the mean lake pH was 6.35 (Table 1). The pH values remained rather stable throughout the year, except during spring melt. Average Gran alkalinity and base cation concentration (Ca + Mg) were 106.5 and 163.2 μequiv./L, respectively, and these

**Fig. 3.** Weekly alkalinity (A), Ca + Mg (B), sulphate (C), and nitrate (D) lake concentrations at Laflamme Lake between June 1981 and May 1992.



variables showed considerable seasonal variations (Figs. 3A and 3B). During spring snowmelt, alkalinity was as low as 40 µequiv./L and Ca + Mg concentration was as low as 105 µequiv./L. During summer and fall, mean alkalinity and Ca + Mg concentration were 80 and 146 µequiv./L, respectively, while in winter, when the lake was principally fed by groundwater, mean alkalinity increased to 130 µequiv./L and mean Ca + Mg concentration reached 188 µequiv./L. Alkalinity and Ca + Mg concentration were strongly correlated ($r = 0.77$, $p \leq 0.05$). Calcium and magnesium concentrations were both more than 20 times higher in the lake than in precipitation. The mean sulphate concentration was 85.2 µequiv./L and varied little in any one year, with annual standard deviations varying between 6 and 10 µequiv./L (Fig. 3C). Sulphate concentration was not correlated with any other variable measured in the lake and represented more than 2.8 times the precipitation sulphate concentration. Mean nitrate concentration was 6.69 µequiv./L and levels were highly variable throughout the year (Fig. 3D). Between June and September, nitrate concentration was often below the detection limit (1.4 µequiv./L), gradually increasing during the winter to attain a maximum value (about 29 µequiv./L) at the start of spring melt. The maximum nitrate concentration in the early spring was comparable to the mean nitrate concentration in precipitation.

The time series analysis for lake waters from June 1981 to May 1992 indicated a significant decrease (16%) in Gran alkalinity from 114.2 to 96.4 µequiv./L and in Ca + Mg concentration (2%) from 164 to 160 µequiv./L (Table 2, Fig. 3A and 3B). Between 1986 and 1991, the lake Ca + Mg concentration showed an 8% decrease from 167 to 154 µequiv./L. For certain water-quality variables, a trend reversal was observed during the study period. For example, between June 1981 and May 1986, sulphate concentration increased 24% from 75.5 to 93.4 µequiv./L, and then between June 1986 and May 1992 levels declined 12% to 82.6 µequiv./L (Fig. 3C). In contrast, nitrate concentration first decreased from 8.6 to 4.3 µequiv./L between June 1981 and May 1986, and then increased to 7.1 µequiv./L between June 1986 and May 1992 (Fig. 3D). No trend was detected in pH between June 1981 and May 1992 or between June 1986 and May 1992.

### Characterization of groundwater quality

Water in the three shallow wells was more acidic than the lake water. Groundwater pH, alkalinity, and Ca + Mg concentration increased with the depth of the sample, whereas sulphate concentration decreased (Table 3), attaining a minimum of 56 µequiv./L at 13 m. Mean nitrate concentrations in the 2.8- and 2.9-m wells were higher than those measured in precipitation, in lake water, and in the 0.8- and 13-m wells.

Like the downward trend in lake-water sulphate concentration, a downward trend in groundwater sulphate concentration was observed in the three shallow piezometers from July 1988 to May 1992 (Table 3, Fig. 4A). Increases in nitrate concentration were found from July 1988 to May 1992 in groundwater from two piezometers (2.8 m at $\alpha = 0.05$ and 2.9 m at $\alpha = 0.10$) (Table 3, Fig. 4B), similar to the significant increase in lake-water nitrate concentration. No trends were detected in alkalinity or Ca + Mg concentration in groundwater from the four piezometers between July 1988 and May 1992.

## Discussion

Between 1981 and 1985, the LLW generally received wet sulphate deposition of over 20 kg·ha$^{-1}$·year$^{-1}$, without a statistically significant trend. Sulphate deposition in the LLW subsequently declined until 1991, resulting in a significant decrease in sulphate deposition for the entire study

**Table 3.** Mean groundwater concentrations for five ionic variables and trend results from July 1988 to May 1992 at LLW.

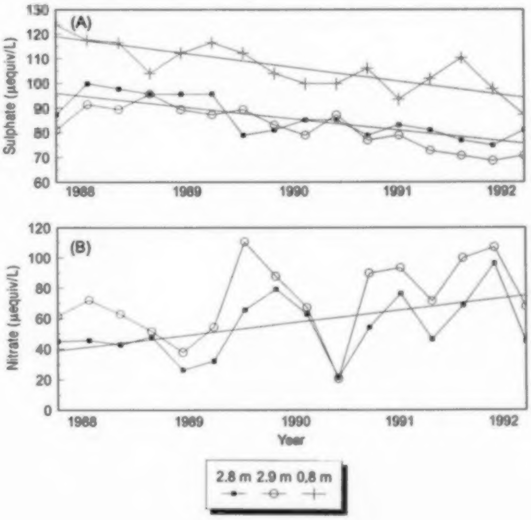
| | Piezometer depth | | | |
|---|---|---|---|---|
| | 0.8 m | 2.8 m | 2.9 m | 13 m |
| pH | 5.2 | 5.9 | 5.9 | 6.8 |
| Alkalinity (μequiv./L) | 12 | 63 | 53 | 856 |
| Ca + Mg (μequiv./L) | 75 | 169 | 169 | 636 |
| $SO_4$ (μequiv./L) | 104 | 86 | 82 | 56 |
| $NO_3$ (μequiv./L) | 5 | 60 | 75 | 1 |
| $SO_4$ | | | | |
| Trend | Negative | Negative | Negative | — |
| Test | Regression | Regression | Regression | |
| Significance level | $\alpha = 0.05$ | $\alpha = 0.05$ | $\alpha = 0.05$ | |
| $NO_3$ | | | | |
| Trend | — | Positive | Positive | — |
| Test | | Kendall | Regression | |
| Significance level | | $\alpha = 0.05$ | $\alpha = 0.10$ | |

period (1981–1991). This phenomenon has also been reported for three sites in Ontario (Dillon et al. 1988; Clair et al. 1995) and at a number of sites in the central and eastern United States (Newell 1993) and linked to decreased $SO_2$ emissions.

In the 1980s, nitrogen oxide emissions remained relatively constant across eastern North America (Sirois 1993). In spite of this, a 19% increase in precipitation nitrate concentration was observed at the LLW. Using a Kernel regression smoother and a geometric model fitted by least squares, Sirois (1993) also observed an increase in precipitation nitrate concentration at the LLW station from 1981 to 1990. Similar increases in nitrate concentration were observed at only a few sites (Bay d'Espoir, Newfoundland, and Caribou, Maine) in North America (Sirois 1993; Clair et al. 1995).

From June 1986 to May 1992, lake sulphate concentration showed a significant decline of 11 μequiv./L. This decrease is linked to the decrease in sulphate concentration both in precipitation falling directly on the lake and in groundwater flow, the two main inputs of sulphate for this lake. The decrease in sulphate concentration in the lake water of the LLW follows a regional trend in nearby lakes (Bouchard 1994). In western Quebec, downward trends in lake sulphate concentration were also recorded, but the magnitude of the decreases was greater (between 27 and 42 μequiv./L; Dupont 1992; Bouchard 1994). For the LLW, a lag exists between the decline in precipitation sulphate concentration (Fig. 2A) and that in lake sulphate concentration (Fig. 3C). Annual sulphate concentration declined substantially after 1985, while mean annual lake sulphate concentration remained stable until 1989. This delay in the response of lake water could be explained by desorption of sulphate from LLW soils following a drop in precipitation sulphate input. Houle and Carignan (1995) demonstrated the existence of a sulphate desorption process in the Bf horizon in the LLW, where 85% of the catchment sulphate is located.

**Fig. 4.** Bimonthly groundwater sulphate (A) and nitrate (B) concentrations from three piezometers in the LLW between July 1988 and May 1992.



Despite the decrease in sulphate concentration in Laflamme Lake, no recovery of lake alkalinity or pH was observed, as occurred in lakes in the Sudbury area of Ontario between 1981 and 1987 (Keller et al. 1992). The response to decreased sulphate may be a combination of changes in base cation levels and alkalinity that can vary over time. From 1986 to 1991, the reduction in sulphate concentration (9 μequiv./L) seemed to be offset by a decrease in Ca + Mg concentration (12 μequiv./L) while during the last year the decrease in sulphate (6 μequiv./L) seemed to be compensated by an increase in alkalinity (4 μequiv./L) (Table 1). A decrease in base cation levels following a decrease in sulphate concentration has been observed in a number of areas (Kelso and Jeffries 1988; Driscoll et al. 1989; Dupont 1992).

Nitrogen-based acidification could partially explain the lack of recovery of lake-water alkalinity. Nitrate-based acidification at the LLW has received little attention because the summer nitrate concentration has always been low and the annual retention of nitrate in the watershed is roughly 78%. Nonetheless, recent trends in nitrate concentration point to an increasing importance of nitrate-based acidification at LLW, e.g., increasing nitrate from June 1986 to May 1992 in lake water and from July 1988 to May 1992 in groundwater measured with two piezometers (2.8 and 2.9 m). Driscoll and Van Dreason (1993) also observed an increase in nitrate concentration that is a major factor explaining a pattern of declining acid neutralizing capacity (ANC) in numerous lakes in the Adirondacks region of New York State.

Nitrates may also play a part in episodic acidification. In spring, at the LLW, the drop in lake alkalinity is mainly due to the dilution of major cations, although the high chronic concentration of sulphate resulting from atmospheric deposition is also a factor. The springtime dilution of major cations was also observed by Molot et al. (1989) in the surface waters of three watersheds in central Ontario. However, at the LLW as well as the three Ontario watersheds, high nitrate concentrations were closely linked to the decline in alkalinity at the start of snowmelt. Decreases in alkalinity and pH caused by high nitrate concentration have been observed at other sites in the northeastern U.S.A. and southeastern Canada as well (Galloway et al. 1987; Peters and Driscoll 1987; Schaefer et al. 1990).

### Conclusion

Precipitation over the LLW showed significant decreases in sulphate concentration and deposition in the last decade, with most of the decline occurring in the latter half of the decade. In contrast to observations made in the Sudbury area of Ontario in the 1980s (Keller et al. 1992), an improvement in pH and alkalinity accompanying the decrease in the sulphate concentration of lake water was not observed at the LLW; in fact, between June 1981 and May 1992, the lake lost 15% of its ANC. The decrease in lake sulphate concentration was compensated by a decrease in Ca + Mg concentration. The gradual loss of ANC demonstrates that the acid inputs to the lake were still too high for a recovery to occur. At LLW, an increasingly important nitrate-based acidification could be partially undermining the positive effects of a decrease in atmospheric sulphate deposition on the recovery of lake-water ANC. In the context of the $SO_2$ and $NO_x$ reduction programs, it seems that some sensitive aquatic ecosystems are still not adequately protected, and additional reduction effort will probably be required in the near future.

## Acknowledgements

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# Effort control in the New England groundfish fishery: a bioeconomic perspective

**William J. Overholtz, Steven F. Edwards, and Jon K.T. Brodziak**

**Abstract**: A multispecies model of the New England fishery was developed to investigate selected biological and economic implications of effort control on groundfish. Performance measures, such as catch, spawning stock biomass, and catch per unit effort, as well as harvest revenue and consumer surplus were used to compare and contrast different levels of effort. Results suggest that significant gains in biological and economic benefits are possible, but major reductions in effort and some short-term losses in catch would be required. Large increases in catch per unit effort on the order of 3–5 times current values would occur, especially for flatfish and haddock. Additional revenues of approximately $10–13 million (U.S.) per year would accrue after several years with overall reductions in effort on this complex of stocks. Changes in stock age-structure would contribute to additional revenues because of the interaction of fish size, price, and increased biomass. Consumers would eventually profit from the lower prices that accompany increased landings. Additional benefits may be obtained after stock rebuilding occurs by application of management measures that stockpile biomass in the initial years of a planning horizon and harvest accumulated stock later.

**Résumé** : Nous avons élaboré un modèle plurispécifique de la pêche en Nouvelle-Angleterre pour examiner certains effets biologiques et économiques de la limitation de l'effort de pêche du poisson de fond. Des mesures de la performance, comme les prises, la biomasse reproductrice et les captures par unité d'effort, ainsi que le revenu de l'exploitation et le surplus du consommateur ont servi à comparer et à opposer différents niveaux d'effort. Les résultats indiquent qu'il est possible d'obtenir des gains notables sur le plan biologique et économique, mais que de fortes réductions de l'effort, et certaines pertes à court terme au niveau des captures, s'imposent. De fortes augmentations des captures par unité d'effort, de l'ordre de 3 à 5 fois la valeur actuelle, se produiraient, particulièrement en ce qui concerne les poissons plats et l'aiglefin. Un supplément de revenus de l'ordre de 10 à 13 millions $US par an est prévisible après plusieurs années de réduction globale de l'effort visant ce complexe de stocks. Les changements dans la structure d'âge des stocks contribueraient à accroître les revenus à cause de l'interaction de la taille des poissons, des prix et de la croissance de la biomasse. Les consommateurs tireraient profit de la baisse des prix qui accompagnerait l'augmentation des débarquements. On peut attendre des bénéfices supplémentaires après le rétablissement du stock grâce à l'application de mesures de gestion qui permettent de faire des réserves de biomasse pendant les premières années de l'horizon de planification, avec exploitation ultérieure du stock accumulé.

[Traduit par la Rédaction]

## Introduction

Georges Bank and bordering regions comprise a very diverse, dynamic, and productive ecosystem located off the eastern coast of the U.S.A. and Canada (Fig. 1). High primary production, fed by near-constant vertical mixing of nutrients, drives the dynamics of this important fish production system (Cohen et al. 1982). Historically, a large biomass of commercially important demersal and pelagic fishes was present in the area, but overfishing by large foreign fleets in the late 1960s and early to mid 1970s caused major declines in these once-prominent commercial resources (Clark and Brown 1977). More recently, pelagic resources such as Atlantic herring and Atlantic mackerel have recovered because of light fishing pressure, but important groundfish resources, overexploited in the late 1970s and 1980s by U.S. and Canadian fleets, remain chronically overfished (Anthony 1990; Northeast Fisheries Science Center 1991*a*, 1992*b*; Canadian Atlantic Fisheries Scientific Advisory Committee 1992; National Marine Fisheries Service 1992; Anthony 1993).

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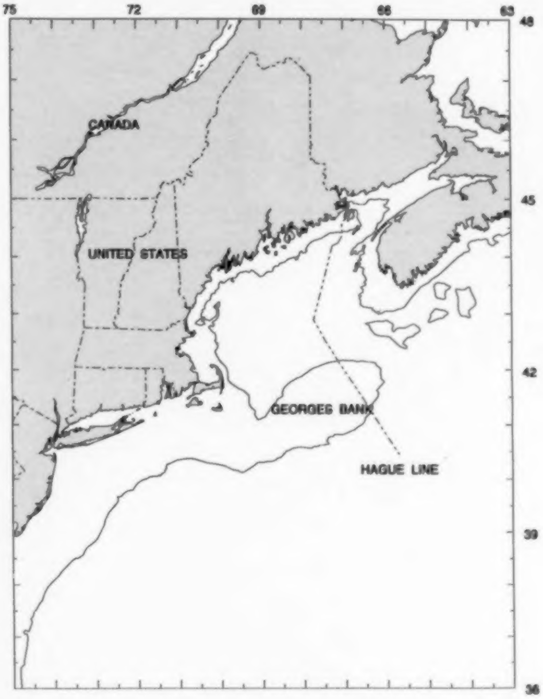
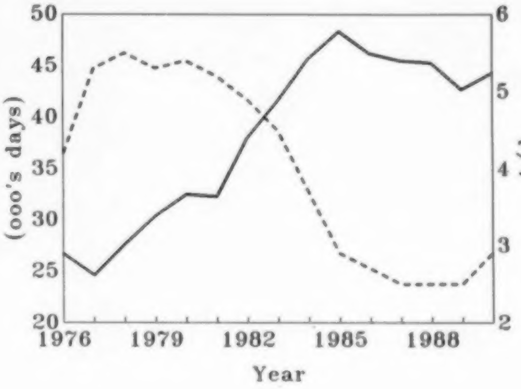
**Fig. 1.** Location of Georges Bank and continental shelf off eastern Canada and the U.S.A. The Hague line separates U.S. and Canadian waters.



Depletion of groundfish resources in the region has followed the classic pattern for uncontrolled access (Gordon 1954; Clark 1990). Otter trawl effort from the U.S. domestic fleet increased steadily on groundfish from the mid 1970s to the mid 1980s and stabilized at relatively high levels, while catch per unit effort (CPUE) declined dramatically during this period (Fig. 2). Effective effort probably continued to increase in the late 1980s because of technological improvements in the fishing fleet and increased effort from other gears such as gill nets, but these increases remain unquantified (Northeast Fisheries Science Center 1991*a*). Total groundfish landings declined dramatically until the mid 1970s, increased somewhat in the late 1970s and early 1980s, and have declined steadily since that time (Northeast Fisheries Science Center 1992*b*). Landings of groundfish have been dominated by cod in recent years, although several flatfish stocks and Georges Bank haddock comprised a large percentage of landings in the 1960s and early 1970s (Fig. 3).

Recent attempts by the New England Fishery Management Council to manage the groundfish fishery in this region have centered on reductions in the number of days at sea as a means of reducing fishing effort, and therefore mortality (New England Fishery Management Council 1993). Edwards and Murawski (1993) used a surplus-production model to estimate that fishing effort should be reduced by 50–70% to maximize the net economic value of this fishery resource. The objective of our study was to examine the relative benefits of reductions in fishing effort in the New England groundfish fishery in greater detail through the use of Monte Carlo simulation techniques and a dynamic age-structured model. The model allowed us to evaluate the impact of changes in effort on biological variables such as catch, spawning stock biomass (SSB), and CPUE, and on economic measures such as harvest revenue and consumer surplus.

**Fig. 2.** Trawl effort in thousands of days (solid line) and CPUE in metric tons per day fished (broken line) for 1976–1990.



## Model overview

A multispecies technological model of the groundfish fishery for the eight primary stocks (Georges Bank cod (*Gadus morhua*), Gulf of Maine cod, Georges Bank yellowtail flounder (*Pleuronectes ferrugineus*), southern New England yellowtail flounder, Georges Bank haddock (*Melanogrammus aeglefinus*), Georges Bank winter flounder (*Pleuronectes americanus*), Gulf of Maine witch flounder (*Gyptocephalus cynoglossus*), and Gulf of Maine American plaice (*Hippoglossoides platessoides*)), found off the New England coast was used to estimate the changes in selected biological and economic performance measures that might occur if fishing effort were changed from current levels. Multispecies technological models are useful for investigating the impacts of changes in fishing effort, fleet dynamics, changes in mesh size, and other factors. Members of the other class of multispecies models, biological models, are used to explore the impacts of mechanisms such as predation and competition on assemblages of species. High fishing rates have been implicated as the major cause of the decline of groundfish resources in the region (Northeast Fisheries Science Center 1992*b*); therefore, a technological modeling approach was warranted, notwithstanding the importance of other factors. Because U.S. otter trawlers account for the vast majority of landings in the region (Northeast Fisheries Science Center 1992*b*), only this segment of the fishery was simulated; Canadian otter trawl effort was held constant in this study. There was no attempt to model fishing fleets, fleet behavior, vessel categories, switching of effort, or other factors; only days fished were modeled in this study.

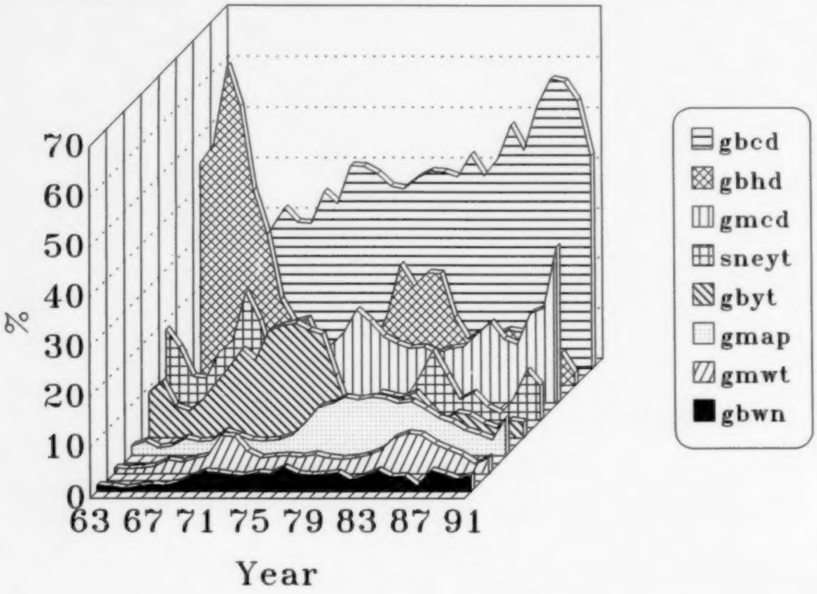


**Fig. 3.** Percentage of total landings by stock for 1963–1991 for the eight primary groundfish, Georges Bank cod (gbcd), Georges Bank haddock (gbhd), Gulf of Maine cod (gmcd), Southern New England yellowtail flounder (sneyt), Georges Bank yellowtail flounder (gbyt), American plaice (gmap), witch flounder (gmwt), and Georges Bank winter flounder (gbwn).

**Table 1.** Parameters from Ricker stock recruitment estimations (where *a* is a slope parameter, *b* is a shape parameter, and *c* is a compensatory parameter) used in the multispecies model of East Coast groundfish for Georges Bank (GB), the Gulf of Maine (GOM), and Southern New England (SNE) stocks.

| Species | *a* | *b* | *c* | Variance | *N* |
|---|---|---|---|---|---|
| GB cod | 0.64 | 1.0 | −0.01 | 0.65 | 10 |
| GOM cod | 0.53 | 1.0 | −0.03 | 1.50 | 7 |
| GB yellowtail flounder | 3.94 | 0.7 | −0.02 | 1.05 | 17 |
| SNE yellowtail flounder | 3.33 | 0.7 | −0.03 | 1.24 | 17 |
| GB haddock | 0.22 | 1.0 | −0.01 | 1.62 | 18 |
| GB winter flounder | 1.26 | 0.7 | −0.04 | 0.48 | 7 |
| GOM witch flounder | 1.43 | 0.7 | −0.03 | 1.14 | 8 |
| GOM American plaice | 3.02 | 0.7 | −0.02 | 0.66 | 7 |

The model is age structured, incorporates simulated measurement error in the estimation of age-specific stock sizes, has species-specific stock recruitment functions with error, and utilizes species-specific and size-specific price models. Model outputs were based on 500 Monte Carlo simulations of the system over a 20-year time horizon beginning in 1991. Model results are reported as averages over the 500 simulations and can be characterized as strategic because they describe the expected future behavior of the system. This model is a useful improvement over previous bioeconomic studies (i.e., Edwards and Murawski 1993) because detailed species-specific simulation results covering a variety of important topics are produced.

Simulations were completed for 11 constant levels of effort ranging from +30 to −70% of the current effort level (45 000 d; Northeast Fisheries Science Center 1992*b*) in 10% increments to allow for a wide range of responses. Selected results are presented that summarize trends common to all eight stocks.

## Model dynamics

The basic model was similar to many common age-structured models of fishery population dynamics (Walters 1969; Sissenwine 1977) and generalized to a multispecies system. The control variable of interest was fishing effort (i.e., days fished) applied to each stock via

$$[1] \quad F_{ij} = q_{ij} f_i$$

where $F_{ij}$ is the stock-specific fishing mortality rate, $q_{ij}$ is the species-specific catchability coefficient, $f_i$ is annual effort in total days fished, *i* indexes the year, and *j* indexes the stock. Catchability coefficients ($q_{ij}$s) were estimated from standardized effort series (Northeast Fisheries Science Center 1991*a*) using regression. All $R^2$ values for model fits were greater than 0.85, and all *q* values were significant ($P \leq 0.01$). Instantaneous fishing mortality rates were estimated from virtual population analysis (VPA) (Gulland 1966; Gulland and Boerema 1973) or modified DeLury models (Collie and Sissenwine 1983) for each stock. Because total effort was the control variable in the model, it was apportioned to each stock area (i.e., Georges

**Table 2.** Parameters used in the price models for the six species in the multispecies groundfish model, where *d* is the intercept, *g* is a total landings parameter, *h* is a size parameter, and *k* is a time indicator parameter.

| Species | *d* | *g* | *h* | *k* | *F* | *P* | $R^2$ | *N* |
|---|---|---|---|---|---|---|---|---|
| Cod[a] | 0.596 | −0.000 95 | 0.0309 | 0.0109 | 26.89 | 0.0001 | 0.666 | 59 |
| Yellowtail | 0.501 | −0.001 59 | 0.2237 | 0.0261 | 24.03 | 0.0001 | 0.706 | 34 |
| Haddock | 0.937 | −0.002 43 | 0.1090 | 0.0193 | 86.36 | 0.0001 | 0.866 | 44 |
| Winter flounder | 0.732 | −0.002 37 | 0.1882 | 0.0519 | 136.79 | 0.0001 | 0.874 | 63 |
| Witch flounder | 0.612 | −0.003 59 | 0.4719 | 0.0688 | 42.96 | 0.0001 | 0.791 | 38 |
| American plaice | 0.274 | −0.001 36 | 0.2125 | 0.0491 | 68.19 | 0.0001 | 0.840 | 43 |

[a] An additional size parameter on weight-squared equal to −0.001 35 was necessary for modeling cod because the largest fish are actually sold at consistently lower prices than small and medium-sized fish.

Bank, southern New England, and the Gulf of Maine) on the basis of the mean historic percentages of effort by region for the period 1976–1990. The percentage of effort by stock region varied little, approximately +5%, over the time period (Northeast Fisheries Science Center 1991*a*).

Age-specific survival was calculated from a negative exponential relationship as

$$[2] \quad N_{1_{ij}} = N_{0_{ij}} e^{-Z_{ij}}$$

where $N_{1_{ij}}$ is the number of fish remaining after 1 year, $N_{0_{ij}}$ is the initial number of fish, and $Z_{ij}$ is the total instantaneous mortality rate.

The Baranov catch equation was used to estimate annual catch in numbers as

$$[3] \quad C_{ij} = N_{0_{ij}} \frac{F_{ij}}{Z_{ij}} (1 - e^{-Z_{ij}})$$

where $C_{ij}$ is the annual catch by species and $F_{ij}$ is the instantaneous fishing mortality rate by species. Fish were assumed to recruit to the fishery at age 2, except for witch flounder, which recruits at age 4. Growth and partial recruitment were assumed to follow patterns from recent assessments (Northeast Fisheries Science Center 1991*b*, 1992*a*). Initial stock sizes were taken from recent stock assessments (Northeast Fisheries Science Center 1991*b*, 1992*a*). Assessments for American plaice, Georges Bank winter flounder, and witch flounder were not available so starting stock sizes were estimated with a modified DeLury model using the method developed by Collie and Sissenwine (1983), assuming equal weighting of errors. Natural mortality (*M*) was assumed to be 0.2 for these stocks.

### Stock size error

Variance estimates of stock sizes at age for Georges Bank and Gulf of Maine groundfish stocks indicate that a reasonable range for coefficients of variation (CVs) of age-specific stock sizes would be 20–50% (Northeast Fisheries Science Center 1991*b*, 1992*a*). A value of 30% was used to simulate measurement error on individual annual stock size estimates by generating normally distributed $N(0,1)$ variates and using them as multipliers of the beginning year stock size for each age group. The multipliers were constrained to ±2 standard deviations; higher and lower values would lie outside of a region of reasonable precision for these stocks.

### Recruitment submodel

The recruitment process was modeled using a three-parameter equation discussed by Devanney et al. (1977) and S.B. Saila and E. Lorda (unpublished data). The recruitment submodel for the *i*th stock was

$$[4] \quad R_{ij} = a_j \mathrm{SSB}_{ij}^{b_j} e^{-c_j \mathrm{SSB}_{ij} + E_{ij}}$$

where $R_{ij}$ is the annual stock-specific recruitment; $\mathrm{SSB}_{ij}$ is the spawning stock biomass, estimated from available maturity schedules for each stock (Northeast Fisheries Science Center 1991*b*, 1992*a*); $E_{ij}$, the error term, is a normally distributed random variable with a mean of 0 and constant variance; and *a*, *b*, and *c* are species-specific parameters.

Recruitment for each stock was estimated by fitting the three-parameter stock-recruitment (SR) model in a three-stage process. First, each series of data was examined with time-series procedures to check for autocorrelation (Box and Jenkins 1976). With one exception, none of the series had significant lags, resulting in part from the relatively few data points for each stock. Then each SR model was parameterized with a nonlinear fitting procedure (SAS Institute Inc. 1990). The *b* value, a shape parameter, was fixed at 1.0 for roundfish and 0.7 for flatfish and the *a* and *c* parameters were estimated (Table 1). Typically, parameters from this type of model are not well estimated, and these eight SR fits were no exception. Finally, an error term that was assumed to be a normally distributed $(0,S^2)$ random variable, where $S^2$ is a constant species-specific variance, was included in the recruitment submodel. The procedure used for estimating the parameters of the error terms was to utilize the residual variance from the SR model fits in a white-noise process (Sissenwine et al. 1988).

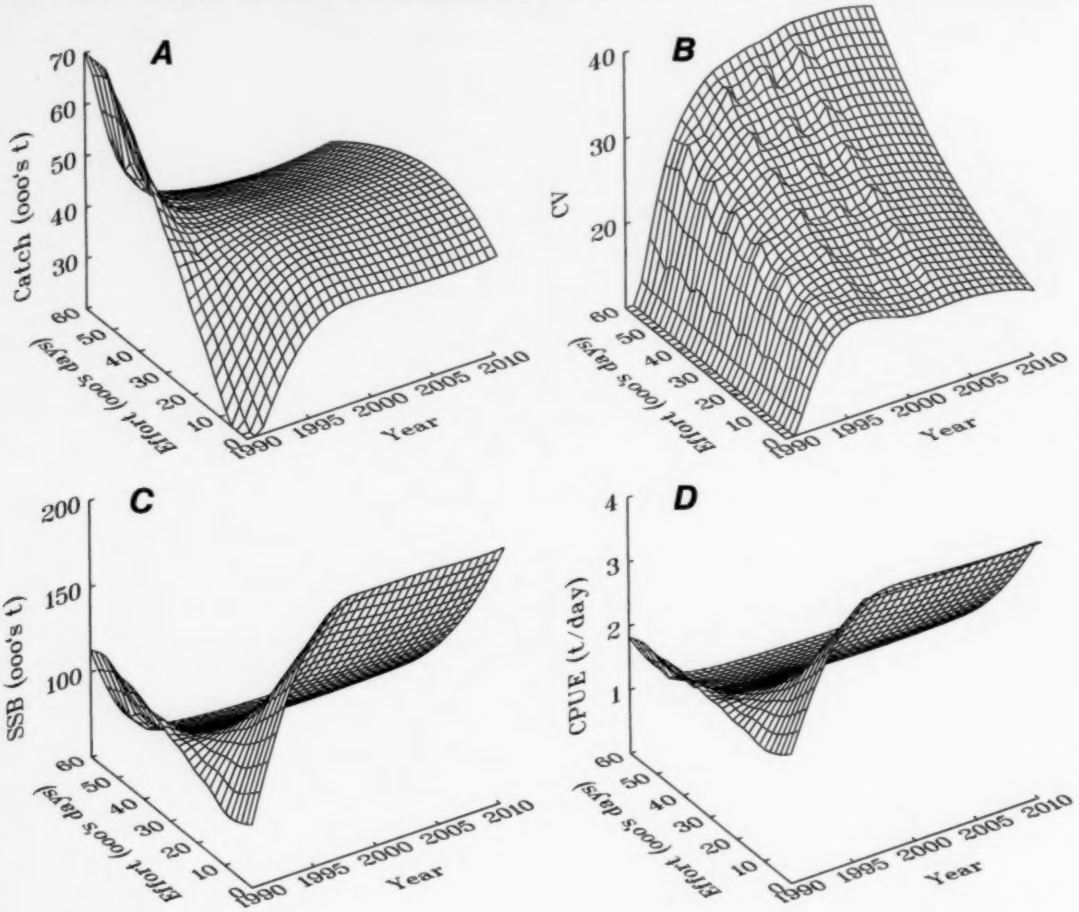
### Price submodel

Time-series models of landings prices of the six groundfish species were specified as follows:

$$[5] \quad P_{jk} = d + gL + hW_{jk} + kT$$

where $P_{jk}$ is the northeast average market price (dollars per pound; 1 pound = 0.454 kg) by species (*j*) and weight category (*k*), *L* is the total landings of the principal groundfish species (millions of pounds), $W_{jk}$ represents average fish weight (pounds), *T* is a time indicator variable (1 in 1972, 2 in 1973, etc.), and *d*, *g*, *h*, and *k* are species-specific

**Fig. 4.** Mean catch of Georges Bank cod (A), coefficient of variation of catch (B), spawning stock biomass (C), and CPUE (D) for the period 1991–2010 under different levels of effort.



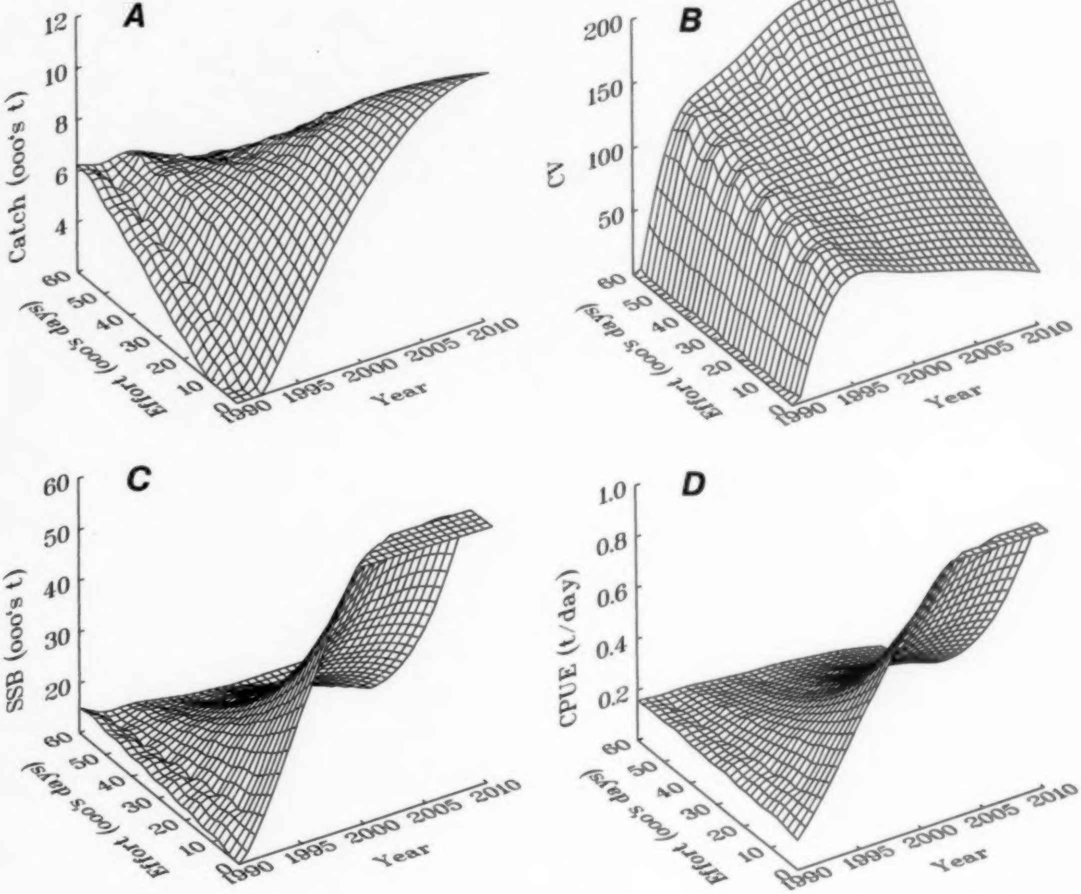
parameters. Three weight categories ($W_{jk}$) were defined (small, medium, and large) with the small category composed of ages 2 and 3, medium ages 4 and 5, and large age 6+ fish of each species. This classification corresponded roughly to the age distribution of commercial size categories commonly used for these species. Average fish weight by species-size category was estimated using the simulated catch at age and mean weight at age each year. A price was estimated for each species-size group. Total revenue for a species was the product of price and catch in weight summed over the three species-size categories.

The price models were fit using ordinary least squares on market data from the years 1972–1990 (White 1987). Nominal prices were adjusted to constant 1990 dollars using the gross domestic product implicit price deflator. Weight was calculated from length–weight relationships for the parameter estimations. Landings of other stocks and species were prorated to the harvest of stocks specifically modeled in this study using proportions during 1988–1990.

Specification of total groundfish landings is consistent with earlier work by Bockstael (1976) that set the standard for groundfish demand analysis and by Gates (1974) and Wang and Chou (1987) who examined the effects of fish size on prices. Unlike earlier work, however, other determinants of prices that are found in structural models were not explicitly specified. Given the emphasis on forecasting prices, we felt it was impossible to also forecast values for regressors such as groundfish imports, consumer income, and preferences for and prices of substitutes. Instead, $T$ was specified as an instrument for the net influence of all factors that were correlated with time during 1972–1990.

All price models were significant at the 0.0001 level, and had $R^2$ values ≥ 0.67 (Table 2). All parameter estimates were significant at the 0.01 level except intercepts in the American plaice and witch flounder models that were significant at the 0.10 level. Because inflation was already removed from the 1972–1990 prices, all results were discounted at a 4% rate when calculating present values.

**Fig. 5.** Mean catch of Georges Bank haddock (A), coefficient of variation of catch (B), spawning stock biomass (C), and CPUE (D) for the period 1991–2010 under different levels of effort.



## Results

### Biological

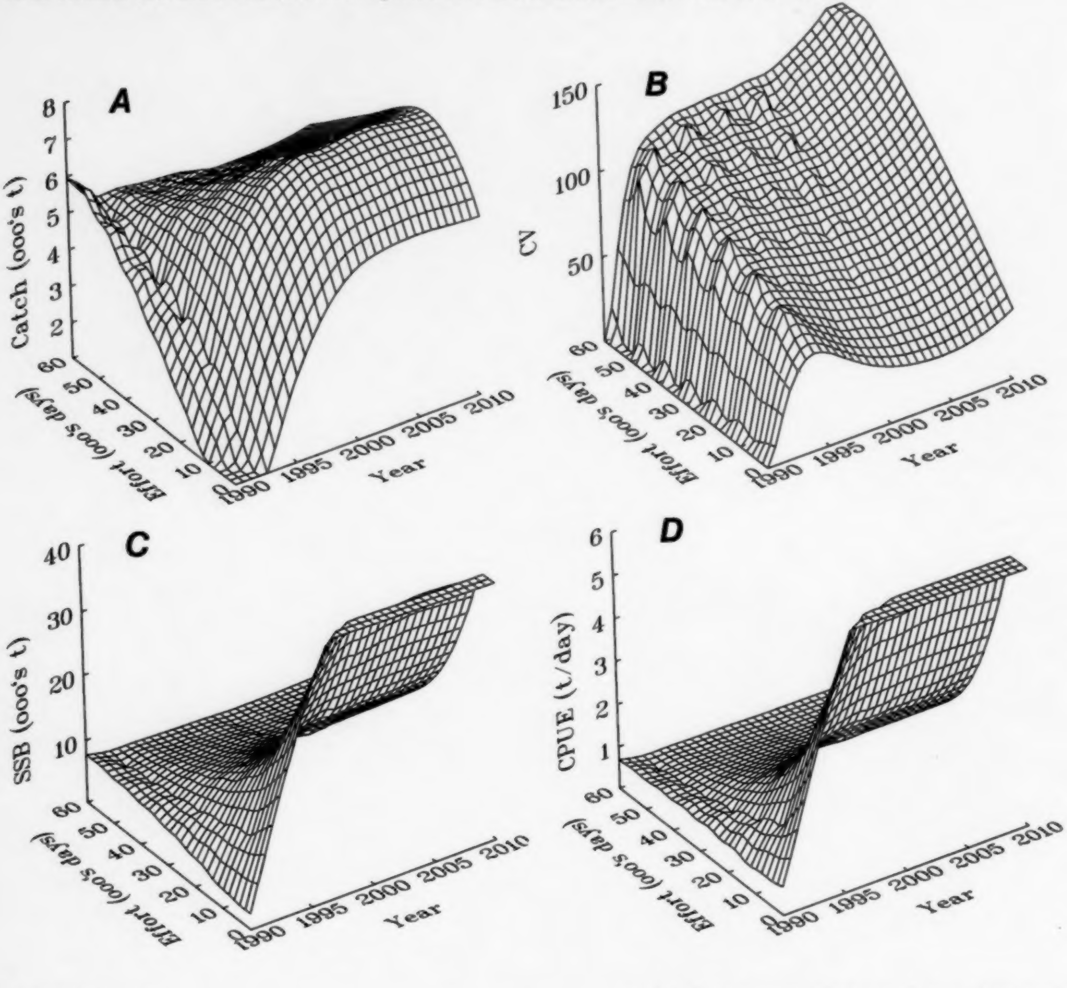
*Catch*

Catch of the entire complex of groundfish stocks declined, sometimes sharply, for several years following significant cuts in effort compared with the status quo (45 000 d), and generally increased within 3–5 years after this initial period. Annual yields for Georges Bank cod, for example, eventually increased by 5000–6000 metric tonnes (t) at lower effort levels following effort reduction (Fig. 4A). Catches of Georges Bank haddock declined initially, then increased steadily after about 3 or 4 years under effort cuts of 30% (31 500 d) or greater, and were an average of 3000–4000 t higher than under the current effort pattern (Fig. 5A). Yields were on the order of 2000 t larger at effort levels of 20 000 – 35 000 d fished for southern New England yellowtail flounder (Fig. 6A). American plaice catches were on average 2000–3000 t larger at effort levels that were 30–50% lower than the status quo (Fig. 7A).

*CV of catch*

CVs of catch were considerably lower on this complex of groundfish stocks when effort was reduced when compared with values for the current level of effort. For Georges Bank cod and haddock, southern New England yellowtail flounder, and Gulf of Maine American plaice, yield CVs were much lower if effort was reduced substantially (Figs. 4B, 5B, 6B, and 7B). For example, the CV for Georges Bank cod would average roughly 22% at 13 500 d as opposed to 35% at 54 000 d (Fig. 4B). CVs for the Georges Bank haddock catch would be 85 and 140%, respectively, for the same effort levels (Fig. 5B). The CV of catch for southern New England yellowtail flounder and American plaice would be similarly lower if fishing effort was reduced (Figs. 6B and 7B).

**Fig. 6.** Mean catch of southern New England yellowtail flounder (A), coefficient of variation of catch (B), spawning stock biomass (C), and CPUE (D) for the period 1991–2010 under different levels of effort.

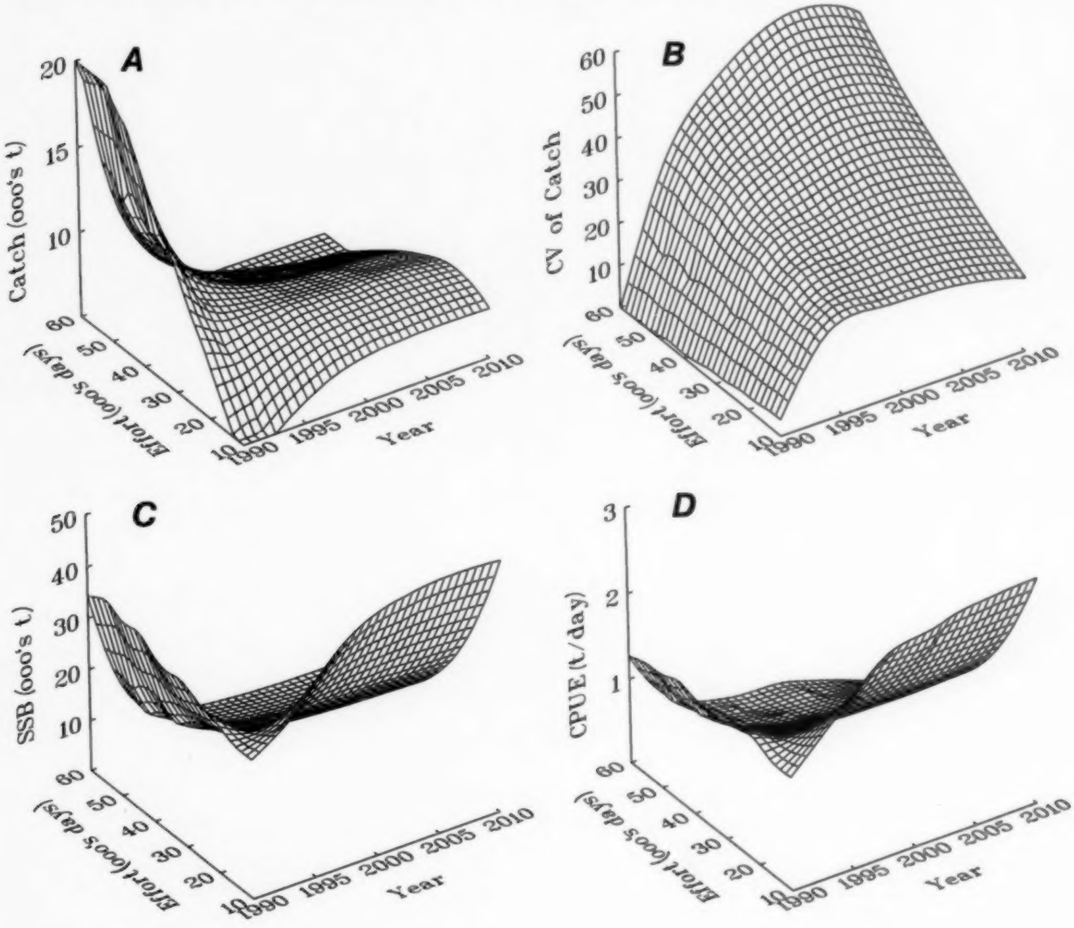


*Catch frequency*
Catch frequencies, summaries of the catch distribution for 500 model runs, for the status quo and −50% options, showed that the catch distributions for all the species had a right-hand skew when effort was lowered. Thus, there was a tendency for catches to be higher in the long term under schemes that reduced effort (Figs. 8A–8D). Georges Bank cod, for example, has a mean catch of 46 000 t and an 80% confidence interval (CI) of 32 815 – 59 558 t under the −50% option, while the mean for the status quo was only 41 127 t with a CI of 26 204 – 57 398 t (Fig. 8A). American plaice catches showed a similar pattern with a mean of 9061 t and 80% CI of 6055 – 12 551 t for the 50% reduction and a mean of 6468 t with a CI of 3311 – 10 208 t for the current level of effort (Fig. 8D).

*Spawning stock biomass*
Following reductions in effort, SSB generally increased steadily for several years, then more slowly for all of the stocks in the groundfish complex. SSB increased substantially for Georges Bank cod and haddock, southern New England yellowtail flounder, and Gulf of Maine American plaice when fishing effort was curtailed (Figs. 4C, 5C, 6C, and 7C). SSB for southern New England yellowtail flounder, for example, nearly doubled under a 50% effort cut, averaging 17 000 t over a 20-year period whereas the status quo was 9000 t (Fig. 6C). Similarly, SSB for Georges Bank haddock more than doubled to 35 000 t over the period for the same 50% reduction in effort versus a 15 000 t average for the status quo (Fig. 6B). SSB for all of the stocks would remain relatively low at current levels of effort, but would improve measurably if effort was reduced (Figs. 4C, 5C, 6C, and 7C).

*SSB frequency*
In all cases examined, SSB frequencies showed that lower effort produces, on average, larger levels of SSB than the status quo (Figs. 9A–9D). For example, the SSB distribution

**Fig. 7.** Mean catch of Gulf of Maine American plaice (A), coefficient of variation of catch (B), spawning stock biomass (C), and CPUE (D) for the period 1991–2010 under different levels of effort.



for the Georges Bank cod stock was roughly 45 000 t larger on average for a 50% reduction in effort (Fig. 9A). The mean SSB for this stock was 114 950 t with an 80% CI of 82 195 – 149 900 t for the −50% effort scenario, and 69 771 t with an 80% CI of 45 315 – 96 910 t for the status quo level of effort. Likewise, the mean SSB for Georges Bank haddock was larger (34 884 t; 80% CI 4439 – 79 845 t) and much more skewed toward larger values than the status quo (14 713 t; 80% CI 791 – 38 830 t), indicating a larger average SSB and range in SSB (Fig. 8B).

### *Catch per unit effort*

CPUE under reduced effort conditions was always larger than that at current effort levels (Figs. 4D, 5D, 6D, and 7D). CPUE for American plaice, for example, nearly doubled to 0.9 t/d with a 30% (31 500 d) decrease in effort and nearly tripled to 1.4 t/d with a 50% (22 500 d) decrease when compared with the status quo (Fig. 7D). For southern New England yellowtail, CPUE would increase to 4.6 times the current value with a 70% decrease in fishing effort (Fig. 6D). Similar patterns were observed for Georges Bank cod and haddock (Figs. 4D and 5D).

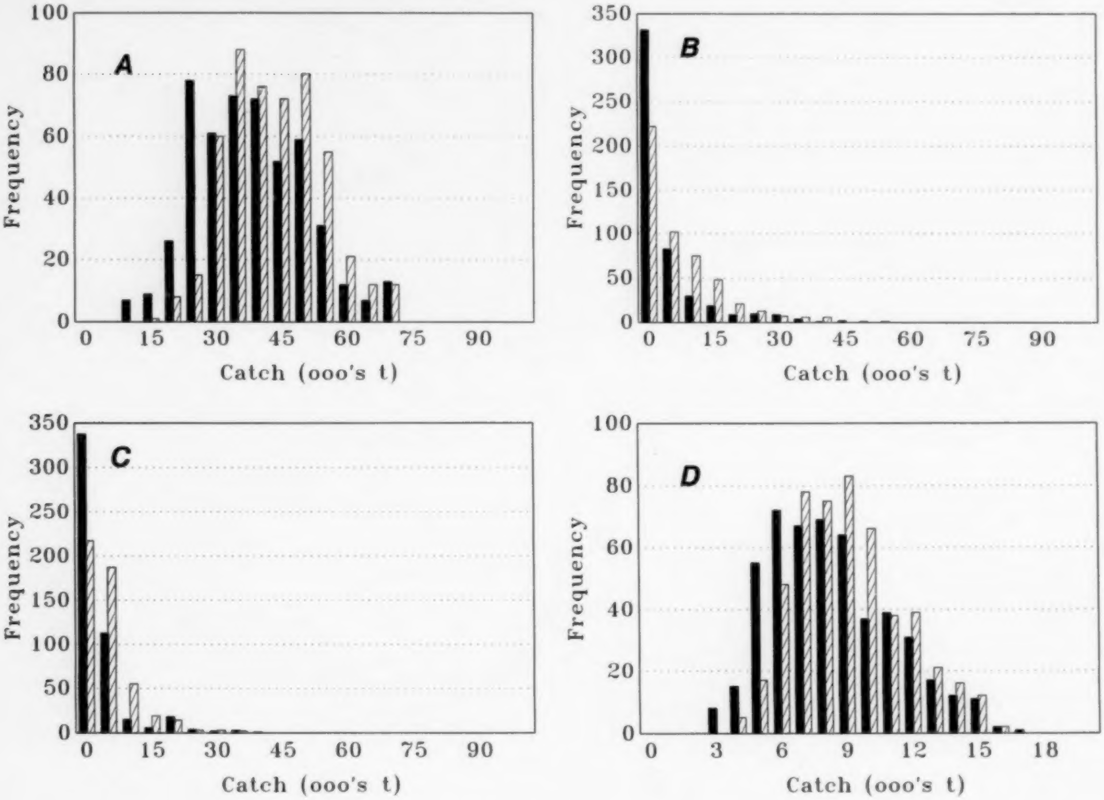
## Economic

### *Revenue*

Total revenue from harvest of the eight groundfish stocks is the product of simulated catches and prices calculated from eq. 5 that were summed across species and size categories. Relative to benchmark conditions in 1991, a 30% reduction in fishing effort initially caused revenue to drop by $10 million, but the loss narrowed during the next few years while stocks rebuilt (Fig. 10). After year 3, revenue exceeded current conditions, climbing to nearly a $10 million increase over the revenue at benchmark conditions after about 10 years (Fig. 10).

Results from the 50% effort reduction scenario were qualitatively similar. Beginning with nearly a $28 million loss in revenue, it took 4 years before revenue exceeded that at current conditions. However, revenue surpassed that in

**Fig. 8.** Frequency distributions of catch ($N = 500$ model runs, solid bars represent the status quo and hatched bars a 50% reduction in effort) for Georges Bank cod (A), Georges Bank haddock (B), southern New England yellowtail flounder (C), and Gulf of Maine American plaice (D).



the 30% effort reduction scenario by year 6 and increased by \$13 million after 11 years.

Finally, there was little difference in revenue between current conditions and 20% growth in fishing effort during the first 2 years, and therefore revenue from the +20% effort scenario initially exceeded revenue from the 30 and 50% effort reduction cases by about \$10 million and \$28 million, respectively. However, a negative trend in revenues relative to current conditions began in year 3, resulting in an \$8 million loss after about 10 years. Furthermore, revenues in this case ended up being about \$18 million and \$21 million lower than in the 30 and 50% effort reduction scenarios, respectively (Fig. 10).

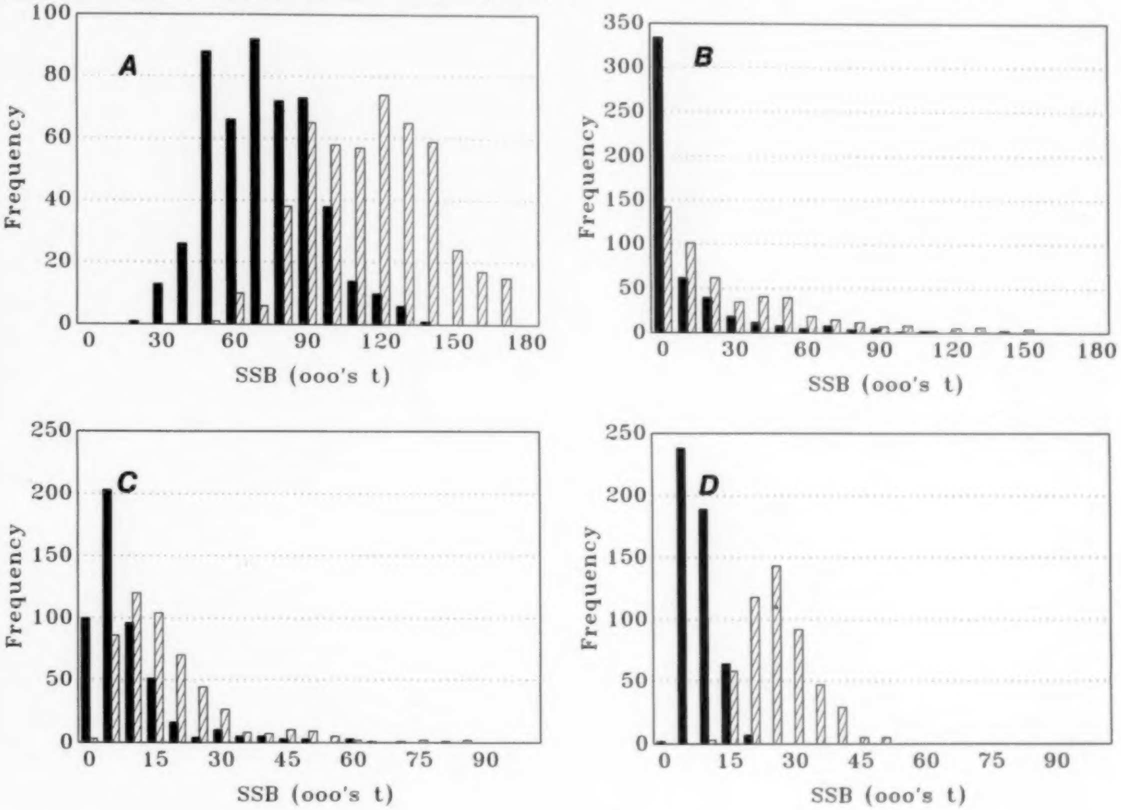
### *Net economic benefits*

Although gross revenue is a key element, evaluating the economic efficiency of effort reduction requires comparisons of four categories of economic value over time: resource rent, producers' surplus, other factor incomes, and consumers' surplus (Copes 1972). Neither the current state nor the effort reduction scenarios, per se, involve property rights to the groundfish resources, so resource rents, or what would be an economic profit for the resource owner(s), are arguably zero in all cases (Cheung 1970; Gordon 1954). Effort reduction could yield resource rent during the transition period of stock rebuilding and beyond if entry was exclusive, such as in some limited entry or individual transferable quota fisheries, but this possibility was not explicitly modelled.

It seems likely that, over time, effort reduction would increase producers' surplus (i.e., industry profit adjusted for opportunity costs), or the income that the owners of fishing capital could earn elsewhere in the economy, simply because operating costs decline with reductions in effort whereas revenues increase. However, the economics data required to model technological production functions and cost relationships, including the opportunity costs of vessel owners and the adjustments they might make to minimize the costs of harvesting larger stocks, have not been collected from the New England otter trawl fleet. Similarly, the opportunity costs of crew are unknown, making it impossible to estimate the effects of effort reduction on other factor incomes or payments to crew for their contributions to the economic value of seafood production.

However, the price models make it possible to estimate changes in consumers' surplus because the demand for groundfish catches in landings markets is derived from seafood consumers. Consumers' surplus can be measured

**Fig. 9.** Frequency distributions of spawning stock biomass ($N = 500$ model runs, solid bars represent the status quo and hatched bars a 50% reduction in effort) for Georges Bank cod (A), Georges Bank haddock (B), southern New England yellowtail flounder (C), and Gulf of Maine American plaice (D).



as the difference between the maximum amount of the income that consumers are willing to spend on groundfish rather than do without it and actual expenditures. Figuratively, consumers profit from a gain in consumers' surplus when prices go down, and they lose benefits when prices increase.

Changes in consumers' surplus, which can be estimated by integrating eq. 5 between prices, are displayed in Fig. 11. The loss in catches that result from reducing fishing effort by 30 or 50% in 1991 explains why the change in consumers' surplus is negative during the first few years. Conversely, consumers' surplus increases when fishing effort increases and further depletes groundfish stocks. However, these results are reversed after 4–6 years when the benefits of increased catches, larger fish, and changes in species composition take effect. Over the long term, effort reduction results in about a $5 million gain in consumer's surplus relative to current conditions and an $8 million gain relative to the +20% effort scenario.

*Net present value*

At reduced effort levels, the net present value of revenue was $100–$130 million larger than under current conditions, if assessed over the 20-year time period (Fig. 12). Similarly, consumer surplus would increase by $21 million over this time frame (Fig. 12).

**Individual species prices and revenues**

*Price*

The effects of landings, fish size, and age-structure discussed above are illustrated here for haddock and yellowtail flounder; there are no stock distinctions.

Haddock price was initially higher by $0.20 and $0.40/lb for 30 and 50% effort reductions, respectively, but eventually declined and averaged about $0.05/lb lower than for the status quo and $0.10/lb lower than at the +20% level (Fig. 13A). Price differences for yellowtail flounder were much smaller after the initial years, with only a few cents per pound separating the various options (Fig. 13B). It is important to remember that although prices were lower for the reduced effort options, there were more fish being landed and at larger sizes, hence the increases in revenue that were outlined in previous paragraphs.

*Revenue*

The aggregate revenue streams displayed in Fig. 10 mask the effects of different fishing mortalities that accompany the effort changes on each species. To illustrate the

**Fig. 10.** Annual revenues standardized against current conditions (horizontal line) under three levels of effort (+20%, −30%, and −50%) for 1991–2010.

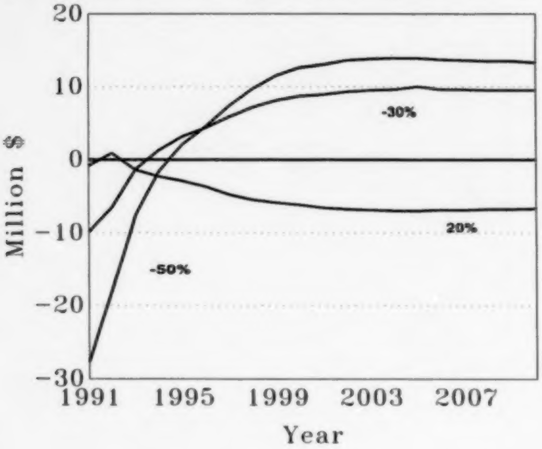
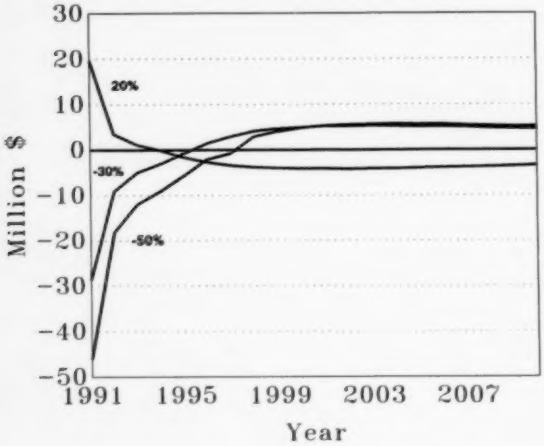


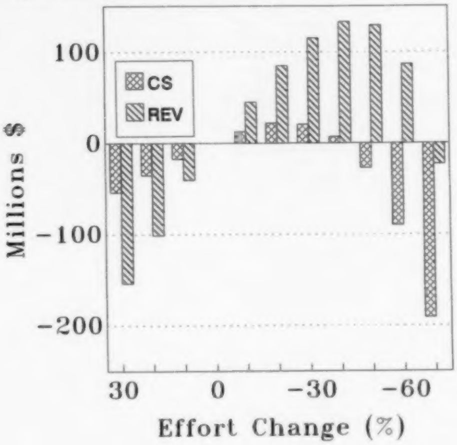
**Fig. 11.** Consumer surplus standardized against current conditions (horizontal line) under three levels of effort (+20%, −30%, and −50%) for 1991–2010.



different species responses over time, results for cod, haddock, and yellowtail flounder are shown in Fig. 14.

Total revenues never recovered for cod at the lower levels of effort necessary for the recovery of the other stocks (Fig. 14A). Cod is the one species whose landings and, hence, revenue never approached status quo conditions when major cuts in effort occurred because fishing mortality never reached former levels. Revenue for haddock, a much depressed stock, on the other hand, increased rapidly following cuts in effort of 30 or 50%, and continued to increase throughout the time series (Fig. 14B). This response suggests that stock rebuilding efforts for haddock are more likely to provide downstream benefits even though the current condition of the stock is extremely poor. Decreases in effort produced revenue increases from 1991 onward for yellowtail flounder (Fig. 14C). This occurred because of a positive price differential in the 1991–1994 period (Fig. 13B), coupled with a larger volume of fish and bigger, higher valued sizes of fish, from 1995 onward. Thus, yellowtail flounder is another species that could provide immediate and sustained increases in revenue if stock rebuilding is initiated.

**Fig. 12.** Net present value of revenue (hatched bar) and consumer surplus (cross-hatched bar), for changes in effort of 10% over the range +30% to −70% for the period 1991–2010. The reference point is the 0 line, representing current conditions.



*Present value*

The present value of revenue for haddock and all of the other flatfish species in the groundfish complex was higher when effort was reduced. Cumulative revenue for haddock was $14 million higher than the status quo, and as much as $83 and $64 million higher for yellowtail flounder and American plaice, respectively, if effort was reduced by 50% (Table 3). Because the cod catch was reduced under effort limitation, cumulative revenue for this species declined (Table 3).

*Scenario modeling*

Results from systematic reductions in effort point to increases in benefits in the outlying years. These results, however, do not take advantage of either the increases in stock size that occur for many species after rebuilding or other options for management that may enhance future benefits. To illustrate this point, several scenarios were simulated that involved modifications in effort different from those previously described. These simulation trials contrast the present value of revenue and consumer surplus from these strategies with the situation in 1991 (strategy 1) and the 50% systematic reduction option (strategy 2) already presented (Fig. 15).

A simple extension of strategy 2, a 50% cut in effort by the U.S. fleet, would be to cut effort in the initial years of the planning horizon and increase effort after the stocks have recovered. Strategy 3 represents a 50% cut in effort

**Table 3.** Present value of revenue by species and effort level for the period 1991–2010.

| | 20% increase | Status quo 1991 | 30% decrease | 50% decrase |
|---|---|---|---|---|
| Cod | 936.7 | 937.2 | 903.4 | 841.5 |
| Yellowtail | 247.2 | 266.4 | 308.2 | 349.6 |
| Haddock | 105.8 | 114.7 | 125.6 | 128.9 |
| Winter flounder | 93.4 | 103.5 | 122.8 | 137.1 |
| Witch flounder | 435.3 | 459.9 | 488.5 | 490.3 |
| American plaice | 317.5 | 354.3 | 403.8 | 418.2 |

from 1991 to 2005 and then a 20% increase until 2010. This strategy increases revenue and consumer surplus compared with strategies 1 and 2 (Fig. 15).

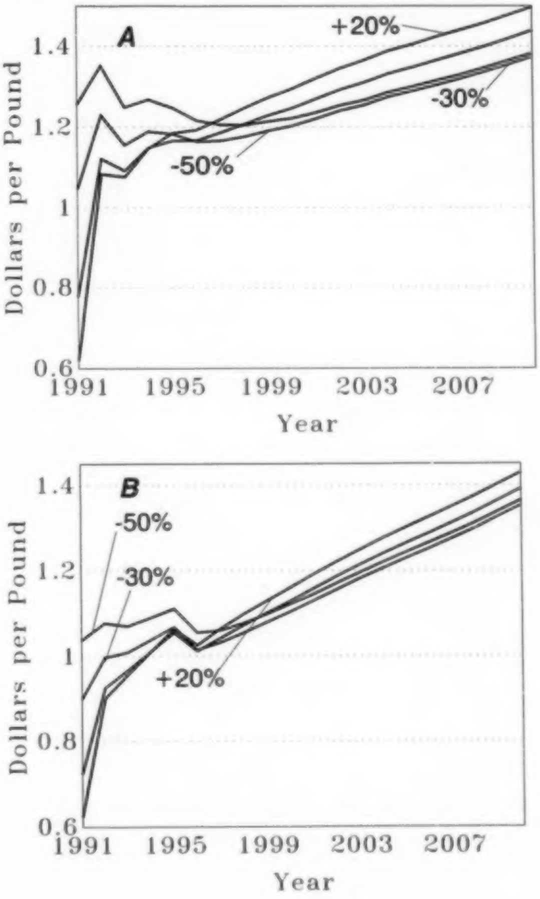
The Georges Bank cod and haddock stocks are fished jointly by the U.S. and Canada, but results presented so far assumed that Canada maintained a constant level of effort. A fishery comanaged by both countries might produce different levels of benefits. Strategy 4 represents joint 50% cuts in effort for both countries (Fig. 15). This strategy results in substantial increases in revenues and consumer surplus, over both the current and the 50% U.S.-only strategies (strategies 1 and 2, respectively) (Fig. 15).

To take advantage of biomass increases that result from decreased effort over time, strategy 5 models a 50% cut in effort by the U.S. and Canada for 1991–2005 and a 20% increase in effort in 2006 for both countries. Again, the benefits increase significantly relative to strategies 1 and 2, primarily because of the increases in biomass that occur in the initial 10 years or so of the series. Resulting individual species biomasses are not heavily affected by this scenario, so rebuilding goals are preserved.

Strategy 6 was produced by simulating a 3-year moratorium on groundfish landings for both countries during 1991–1993, followed by an increase in effort to 22 500 d (50% of the current effort level), and finally a 20% increase in 2006. This essentially repeats strategy 4, but adds the 3-year moratorium on the front end. This approach produces a decrease in revenue, but a large increase in consumer surplus, because of the increases in landings and decreases in prices in the latter years of the projected series (Fig. 15).

Finally, strategy 7 represents a 5-year moratorium during 1991–1995 followed by an increase to 50% of former effort levels. This strategy increases revenue, but decreases consumer surplus, compared with strategy 6 (Fig. 15).

**Fig. 13.** Price of haddock (A) and yellowtail flounder (B) (dollars per pound) at effort levels of +20%, status quo, −30%, and −50% for 1991–2010.



## Discussion

Simulation results, relative to the status quo level of fishing effort, suggest that significant gains in catch, SSB, and CPUE result from reductions in effort of 30–60%. After initial short-term losses, catches improved dramatically for all eight groundfish stocks, and total catches would be up to 15 000 t larger, depending on the extent of systematic effort reductions. More and larger fish would be stockpiled into older age groups of all species, and CPUE would increase greatly, especially for flatfish.

Positive crossover for revenue relative to current conditions occurred 3–5 years after 30 and 50% reductions in effort. Short-term losses in revenues and consumer surplus would be offset by long-term gains, judging from the present value calculations. Revenues would be larger, and consumers would be paying lower prices. It is important to reiterate that the stochastic projections presented here are intended for comparing the relative benefits of effort control strategies and not as predictions of future conditions.

We feel that the results from this modeling exercise could, in general, be conservative for several reasons. Our

**Fig. 14.** Standardized revenue for cod (A), haddock (B), and flounder (C) at effort levels of +20%, status quo, −30%, and −50% for 1991–2010.

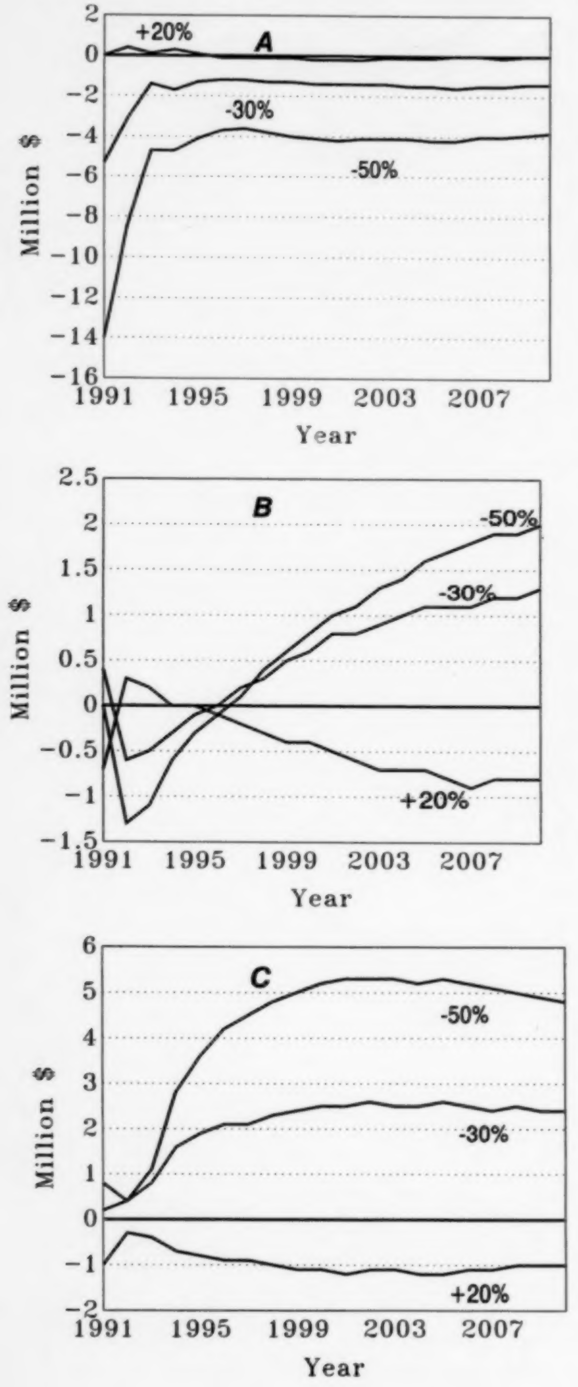
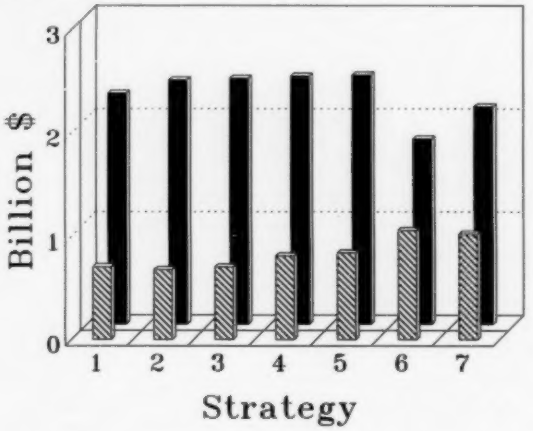


**Fig. 15.** Present value of revenue (solid bars) and consumer surplus (hatched bars) (billions of dollars) for seven effort strategies described in the text for 1991–2010.



model only covers a segment of the total groundfish fishery; therefore, we are probably underestimating benefits. Also, Edwards and Murawski (1993) estimated that benefits on the order of $150 million per year in net economic value are possible with effort reductions of 70% on the entire groundfish system under a much longer term planning horizon.

Our assumption of stationarity in the recruitment process for each stock may have an impact on our predictions of future benefits. Recruitment for most of these stocks has continued to decline on average under high levels of fishing effort; therefore, our prediction of relatively stable catch and SSB under current conditions may not hold, and recruitment may decline further under the status quo.

Alternatively, the benefits of stock rebuilding under reduced effort may be larger than we predict if recruitment improves because of enhanced age-structure and reduced bycatch mortality under a lower effort regime. Overall, the fact that the model results are likely to be conservative further supports our conclusion that much larger benefits would accrue in the long term at lower levels of effort.

The other management scenarios that we investigated suggest that there are probably additional gains to be made by adjusting effort during stock rebuilding, rather than holding effort constant at reduced levels. Although the strategies presented here are not optimizations, they represent commonly available management measures that could affect fishing effort directly. These approaches have either previously been used or are being considered for managing groundfish and other fisheries in the Northwest Atlantic (New England Fishery Management Council 1993). Effort control policies based on optimization would certainly offer an alternative way to examine potential benefit streams from this multispecies system, provided that management goals can be described with an objective function (Hilborn and Walters 1992; Overholtz et al. 1993).

Even though the policies we examined would entail some short-term losses, it appears that long-term benefits would be substantial. Cooperative management with Canada and (or) a fishing moratorium would produce substantially more benefits than the systematic effort reductions presented here. Additional benefits could be gained by taking advantage of increases in stock size and improved recruitment after stock rebuilding occurs, if fishing effort remains light to moderate. If net benefits to the U.S.A. are of paramount importance, simulation results suggest that a fishing moratorium would eventually have a beneficial effect for consumers.

The ability and (or) importance of rebuilding depressed stocks of groundfish is illustrated by the examples of haddock and yellowtail flounder we presented. Substantial benefits would accrue if the stocks of these species were rebuilt even though their current status is extremely poor. Stock rebuilding apparently has an economic basis; benefits are enhanced by reducing effort in this groundfish fishery. Haddock and flatfish are potentially very productive and valuable components of this multispecies system, but their stock dynamics can only be maintained under low to moderate rates of fishing. Yields and recruitment of cod remained somewhat stable under the current intense multispecies trawl fishery, although they have declined more recently (Northeast Fisheries Science Center 1995). The other species have declined dramatically and are currently overfished (Northeast Fisheries Science Center 1992*a*) and economically depressed.

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# Reproductive interactions between sympatric brook and brown trout in a small Minnesota stream

**Peter W. Sorensen, James R. Cardwell, Tim Essington, and Dana E. Weigel**

**Abstract**: The reproductive activities of sympatric brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) in a Minnesota stream were documented on a regular basis for 3 years to determine whether behavioral interactions between these species might influence their spawning activities and success. The spawning seasons of brook and brown trout consistently overlapped by 2–4 weeks, during which time nearly 10% of all sexually active females were simultaneously courted by males of both species. Male brook trout attempted to hybridize most frequently; however, both our behavioral observations and population census suggested that these fish had little success. There was also strong evidence of frequent redd superimposition, particularly by the later spawning and larger brown trout. Analysis of redd site habitat demonstrated that these species had overlapping preferences. Although it seems likely that attempted hybridization and redd superimposition work to the disadvantage of both species, the effects of these activities are likely to be particularly severe for the brook trout, which spawns earlier in the season, is smaller in size, and rarely survives to be old enough to spawn twice. Thus, reproductive interactions may be partially responsible for the displacement of brook trout by brown trout in many regions of North America.

**Résumé** : Nous avons suivi de façon régulière pendant 3 ans les activités reproductrices de populations sympatriques d'omble de fontaine (*Salvelinus fontinalis*) et de truite brune (*Salmo trutta*) dans un cours d'eau du Minnesota pour déterminer si les interactions comportementales de ces deux espèces pouvaient avoir un effet sur leurs activités reproductrices et sur le succès de la fraye. Les saisons de fraye de ces deux salmonidés se recoupaient régulièrement de 2–4 semaines, période pendant laquelle près de 10% de toutes les femelles sexuellement actives étaient courtisées simultanément par des mâles des deux espèces. C'étaient les mâles d'omble de fontaine qui essayaient le plus fréquemment de se croiser; toutefois, tant nos observations du comportement que les dénombrements des populations permettent de penser que ces poissons connaissent peu de succès. On note aussi de nettes indications d'une superposition des nids, particulièrement de la part des truites brunes, plus grosses et à ponte plus tardive. L'analyse de l'habitat des nids montre que ces espèces ont des préférences qui se chevauchent en matière d'habitat. S'il paraît vraisemblable que les tentatives d'hydridation et la superposition des nids jouent en défaveur des deux espèces, les effets de ces activités risquent d'être particulièrement graves pour l'omble de fontaine, qui fraye plus tôt, est plus petit et survit rarement assez longtemps pour pondre deux fois. Les interactions dans la reproduction peuvent donc être partiellement responsable du remplacement de l'omble de fontaine par la truite brune dans de nombreuses régions d'Amérique du Nord.

[Traduit par la Rédaction]

## Introduction

Throughout many areas of central and eastern North America, populations of native brook trout, *Salvelinus fontinalis*, have been declining, apparently as a consequence of the introduction of European brown trout, *Salmo trutta*, whose numbers have been increasing (Kelley et al. 1980; Waters 1983; Fausch 1989). Although a variety of ecological, physiological, and behavioral factors have been hypothesized to be responsible for the apparent displacement of brook trout, no single, clear explanation presently exists (Fausch 1988, 1989). One possibility that has not been rigorously investigated is that because these species spawn at similar times of the year and in similar habitats (Witzel and MacCrimmon 1983), they might be interacting on spawning redds to the detriment of the reproductive success of the brook trout. This possibility seems particularly credible because hybrid brook–brown trout (tiger trout) have

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been noted in many streams (Waters 1983; Witzel 1983). However, hybridization behavior between trout has yet to be described, so it is difficult to evaluate its possible significance. Furthermore, only one study has described the reproductive activities of sympatric brook and brown trout; although it reported little overlap in the locations of the spawning nests (redds) of these species in Ontario (Witzel and MacCrimmon 1983), the behavior of sexually active fish was not systematically monitored. The possibility that fish might be interacting while spawning thus remains unanswered.

The principal objective of the present study was to document the reproductive activities of sympatric brook and brown trout to determine if and how spawning interactions might influence their relative reproductive success and explain the presence of hybrid tiger trout. A small, well-studied Minnesota stream (Valley Creek) that contains sympatric self-sustaining populations of brook, brown, and rainbow trout (*Oncorhynchus mykiss*) was selected for the present study, which sought to answer four related questions. First, when and where do brook and brown trout spawn within this stream, and is there any overlap in spawning times or locations? Second, is spawning activity correlated with particular environmental variables and how might these variables influence spawning activity? Third, how frequently do brook and brown trout interact and (or) attempt to hybridize? Fourth, what is the age and size of sexually active male and female brook and brown trout and could these characteristics influence behavioral interactions and their spawning activity and reproductive success?

## Materials and methods

### Study site

Valley Creek is a short (6 km long), first-order stream flowing in a limestone valley and emptying into the St. Croix River, Minnesota (for map see Elwood and Waters 1969). Its base discharge is about 0.15 $m^3/s$, average stream width is 4 m, and the bottom substrate varies from sand to rubble. The stream flows through private land, is not fished, and has not been stocked for at least 20 years. Our study section is approximately 1 km long and has a pool–riffle morphology with riffles about 10 cm deep and pools 1 m deep. The fish community consists primarily of brook, brown, and rainbow trout, and slimy sculpin (*Cottus cognatus*). This system supports an extremely high biomass of trout (Waters 1988) and has been the subject of many studies including a 20-year investigation of annual fluctuations in trout abundance and composition (Waters 1983).

### Spawning activity

Spawning activity was documented during 1990–1992 beginning the last week of September and continuing through the end of November in each year (dates determined by a pilot study conducted in 1989). Observations were made daily in 1990 and at least every other day in 1991 and 1992. The protocol for documenting spawning activity had two components. First, to gain a cursory understanding of the distribution of spawning trout, we walked along the study section in the late morning looking for sexually active trout and (or) changes in stream bed topography suggestive of redd construction. Next, to determine accurately the nature of suspected spawning activity (a step necessitated by the fact that we frequently disturbed fish during initial assessment) we carefully returned (often on hands and knees) to those areas of the stream with suspected activity to conduct 30-min evaluations of fish activity with the aid of binoculars and (or) a video camera with a telephoto lens. Canvas observation blinds (3 m in length) were constructed on the stream's banks where spawning was observed with some regularity. Locations with active redds were recorded on a map produced from an aerial photograph. An active redd site was defined by the presence of (a) sexually active female(s) and an excavated area in the stream bed. Notes were also kept on areas of the stream that appeared to have had spawning activity (i.e., possible inactive redds) so that we could monitor them for future activity. These data were not used in the present analysis, however. Water temperature was also measured at the head and tail sections of the study area.

A female was considered sexually active if it was seen: digging (displacing gravel to construct a nest with strong flexures of the body while turned on one side), probing (slowly dropping into the nest and pushing her anal fins into the excavation), covering (pushing gravel over eggs in a nest), or holding over a nest (maintaining position) (after Liley et al. 1986*a*). Male reproductive behavior was also noted and was characterized by Liley et al.'s (1986*b*) criteria. Briefly, males were considered sexually active if they were courting or spawning with female(s). Courting males attended females (maintained position behind and to the side of the female), occasionally venturing to their side to quiver (exhibit a low amplitude undulation of their body). Spawning, or the simultaneous release of gametes by males and females, usually occurred after a period of intense quivering and was accompanied by mouth gaping. In most instances, sexually active females were attended by more than one male and a dominance hierarchy among males was noted. We could usually identify individuals within these hierarchies by their size and pigmentation patterns.

Sexually active trout were sampled during the spawning season in 1990 while on their spawning redds to verify species identity and to determine their size, age, and gonadal condition. A stationary electroshocking technique that employed 1-m sections of copper pipe electrodes connected via electrical cord to a portable 220-V (3 A) pulsed DC generator (T&J Manufacturing, Oshkosh, Wis.) capable of being activated by a remote switch. Electrodes were placed 1–2 m upstream and downstream of active redd sites to which fish almost always returned within 1 h if not disturbed further. Prior to collection, spawning fish were observed for 30 min and the behaviors of individual males and females categorized. The fish were then shocked (for 30 s), removed from the stream using nets, lightly anesthetized (0.5% solution of 2-phenoxyethanol), weighed to the nearest gram with a portable scale, and measured (total length (TL) to the nearest millimetre). Gonadal condition was assessed by gently squeezing their abdomens and scale samples from their dorsal body surfaces were removed for aging. Sampled fish were then placed into an aerated bucket

**Table 1.** Year, sampling effort for that year, and number of spawning females observed that year (site-days), first summed over that year, and then broken down by the species composition of the males seen courting the females.

| Year | Yearly sampling effort (days) | Total no. of brook female site-days | Site-days with brook males (%) | Site-days with brook and brown males (%) | Site-days with brook and rainbow males (%) | Total no. of brown female site-days | Site-days with brown males (%) | Site-days with brown and brook males (%) | Site-days with brown and rainbow males (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1990 | 34 | 80 | 95 | 5 | 0 | 75 | 76 | 24 | 0 |
| 1991 | 18 | 26 | 96 | 4 | 0 | 30 | 83 | 17 | 0 |
| 1992 | 24 | 60 | 95 | 4 | 0 | 53 | 81 | 15 | 4 |
| Mean | | | 95 | 5 | 0 | | 81 | 19 | 1 |

of water to recover and released into the stream. Age was later estimated by counting the number of annuli on these scales using a microfiche reader after the scales had been pressed onto an acetate strip (Hanson and Waters 1974). Characteristics of dominant male, subordinate male, and female trout were compared by one-way analysis of variance (ANOVA; SYSTAT, Evanston, Ill.) and if significant differences were found between them ($P < 0.05$), by pairwise follow-up tests using the Tukey procedure (Winer 1971).

After spawning ceased in 1990, previously used redds in the stream were examined and their physical attributes characterized. To evaluate groundwater input the temperature differential between the gravel and overlying stream water was used. This was measured in early December when stream temperatures had fallen below 5°C, creating a marked contrast with the 10°C groundwater (as measured at a spring flowing into the creek). Temperature was measured using an electronic oxygen–temperature probe (Y.S.I. model 57, Yellow Springs, Colo.), which was first held 5–10 cm above the surface of the redd and then inserted to about this depth into the gravel. Water velocity was also measured approximately 5 cm above the center of the redd using a digital flow meter (Marsh-McBirney No. 201, Frederick, Md.). Water depth was measured using a metre stick. Finally, the redd was dug up to ascertain that eggs were present; if they were not, its data were not used (4 of 34 redds did not have eggs). Redd site choice was analyzed by categorizing redds as being used by brook trout only, brown trout only, or females of both species (although not necessarily at the same time) and comparing their attributes by ANOVA as described above.

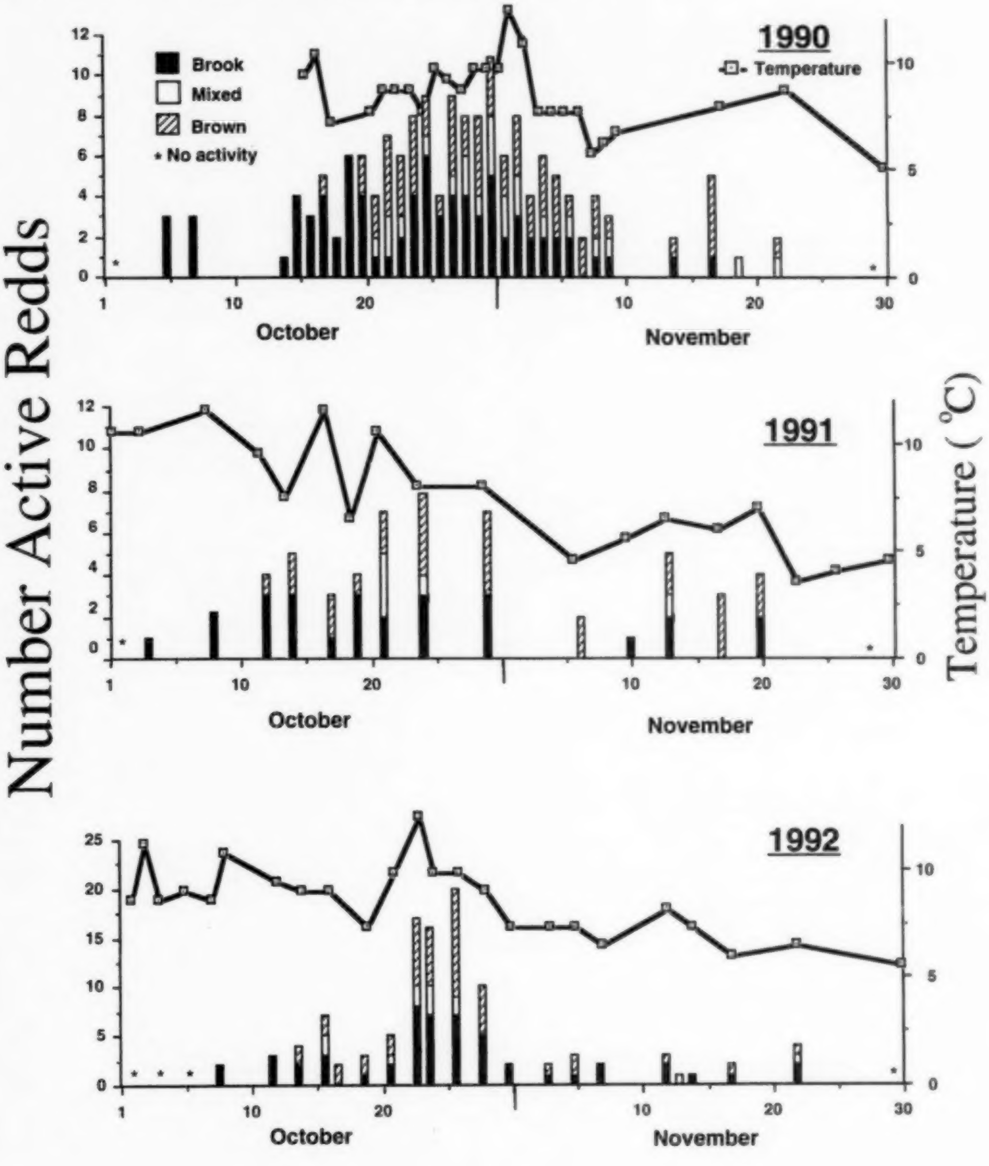
To confirm that the interactions that we had observed between male and female brook and brown trout reflected attempted hybridization, we also conducted a behavioral study using captive mature fish. Mature domesticated brook and brown trout were obtained from a local fish hatchery and held temporarily in 100-m$^2$ plastic mesh cages constructed in Valley Creek in the fall of 1990 (a pilot study using wild fish in 1989 found that wild fish refused to spawn in enclosures). Female brook trout were then induced to ovulate by hormone treatment (two 0.5 mL/kg injections of Oviprim, Syndel, Vancouver, B.C.) and placed individually into circular 1000-L tanks containing gravel and 20 cm deep 10°C water that had been induced to flow in a circular manner using submerged pumps. Fish were given a day to acclimate, after which a male brook or brown trout was moved into the tanks. Behavioral activity was then monitored daily for 4 days after which the gravel was examined for eggs. Eggs were examined under a microscope to determine if they were fertilized. Twelve experiments were conducted, 6 in which male brook trout were placed with female brook trout and 6 in which male brown trout were placed with female brook trout. Female brown trout were not tested because they were not made available to us by the hatchery and because wild brown trout failed to spawn in our tanks.

Finally, to determine the success rate of hybridizing trout, the population of trout in Valley Creek was surveyed to estimate hybrid abundance. This survey was conducted in July 1992 using a boat-mounted 220-V pulsed DC electroshocker (T&J Manufacturing, Oshkosh, Wis.). Captured fish were weighed, measured, and released after receiving a small clip on the pelvic fin. A second pass was then made through the study section a week later and the population estimated on the basis of the numbers of recaptured fish using the Petersen mark–recapture formula (Ricker 1975). A population estimate was calculated on the basis of fish greater than 16 cm TL, a size range that had a high recapture rate of 65% or higher. Previous studies have verified that fish mortality from electrofishing in this manner is less than 1% and that fish move little in this system (Weigel 1994).

## Results

In all 3 years, female brook trout started spawning in early October and brown trout 2–3 weeks later (Fig. 1). Both species then continued spawning through mid-November, resulting in a period of overlap that was at least 3 weeks long. During this time, behavioral interactions were common and approximately 10% of all spawning groups had more than one species of male (Table 1). The vast majority of these mixed-species spawning groups were instances of brook trout males courting female brown trout, but the opposite was also observed on several occasions. Nearly 20% of all sexually active brown trout were courted by male brook trout. Most brook trout seen courting female

**Fig. 1.** The number of spawning redds noted each day that observations were made by year. Brook denotes groups comprised of brook trout only, brown denotes groups comprised of brown trout only, and mixed denotes groups that had more than one species (any combination of sexes or species). Days on which observations were made but no spawning activity was seen are denoted by an asterisk. Daily stream temperature is denoted by the line connecting the square symbols.

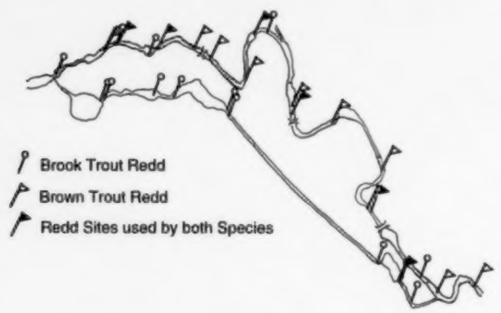


brown trout were present as part of a group of male brown trout, but on several occasions they were the only males present. On two occasions rainbow trout males were also observed courting brown trout females; one did so in the presence of a male brook and brown trout, which it subsequently chased away. That male rainbow trout was seen to spawn (release sperm) with the female brown trout.

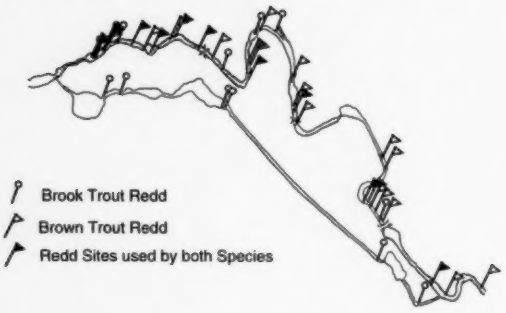
Behavioral interactions between males and females of different species on the spawning redds appeared to be no different than interactions with conspecifics. Dominant male brown trout actively chased brook trout males away; the latter would frequently swim up to females from hiding places in the stream only to be chased away or attacked. On those occasions when brook trout males gained access

**Fig. 2.** The locations of spawning sites in 1990 and 1992, the years with the greatest sampling effort. Site is used to describe a specific location where sexual activity was observed. Some sites thus had more than one redd because of redd superimposition. If a site was used by females of both species either simultaneously or sequentially it was denoted as a site used by both species.

Valley Creek Spawning Sites Oct.-Nov. 1990



Valley Creek Spawning Sites Oct.-Nov. 1992



to female brown trout (generally when the dominant fish was chasing another fish away) they would exhibit quivering behavior. Female brown trout usually appeared to accept the presence of male brook trout but occasionally bit or chased them; however, these females often attacked small brown trout males as well. Although brook trout males were subordinate in all instances in which brown trout males were also present, they did not appear to behave differently towards brown trout females than towards brook trout females; they consistently attempted to approach the female, and if not chased away by a larger (dominant) brown trout (which was generally the case), would quiver alongside the female. A brown trout female was never seen to spawn (release) gametes in the presence of a brook trout male, however. In our hybridization experiment using captive fish, all male brook and male brown trout placed with ovulated female brook trout were seen to be courting and digging redds. All apparently spawned because fertilized eggs were collected in all tanks (survival beyond the experiment was not noted).

**Table 2.** Mean depth, velocity, and temperature differential of spawning redds in 1990 by species.

| Species | $n$ | Depth (cm) | Velocity (cm/s) | Temperature differential[a] (°C) |
|---|---|---|---|---|
| Brook trout | 11 | 44±26[b] | 11.7±10.3 | 0.4±0.4 |
| Brown trout | 12 | 20±5 | 43.0±16.1[b] | 0.3±0.5 |
| Brook and brown trout | 7 | 27±9 | 23.4±17.2 | 0.4±0.5 |

**Note**: Values are given as the mean ± SD.

[a] Temperature above gravel − temperature in gravel; a measure of groundwater upwelling. The mean differential at 10 sites not used for spawning was 0.1 ± 0.1°C.

[b] $P < 0.05$.

Spawning activity was restricted to specific sections of Valley Creek, with large stretches of stream being ignored (Fig. 2). Female trout of both species demonstrated a remarkably strong tendency to choose the same spawning sites each year, and in several instances we knew that redd placement varied by less than 0.5 m between years because of the position of distinctive landmarks on the stream bank. Approximately 25% of all spawning sites were used by both species with the majority of these being instances of brook trout redds being reexcavated by later spawning brown trout. Redd sites used exclusively by brook trout females were in deeper and slower flowing water than those chosen by brown trout females ($P < 0.05$). Both species chose regions where gravel temperatures exceeded the temperature of the overlying water, suggesting that the sites had groundwater input (Table 2). Locations used by both species had intermediate water depths and velocities.

The average age of sexually active brook trout females was just over 2.0+ years with animals ranging in age from 1.0+ to 3.0+ years (Table 3). On average, male brook trout matured slightly earlier than female brook trout and several spermiated males less than 1 year of age were captured. Although these precociously mature animals were subordinates, we lack the data needed to determine whether they were "sneakers." Dominant male brook trout tended to be larger and older than subordinate male brook trout, but these differences were not significant ($P > 0.05$). In contrast, dominant male brown trout were significantly older and larger than subordinate male brown trout ($P < 0.05$). Two dominant male brown trout were 8 years old, 5 years older than the oldest brook trout. Although sexually active female brown trout were, on average, older (3.0+ years) than sexually active brook trout females (2.4+ years), it was clear that few females of either species lived long enough to spawn twice. Female brown trout were also larger than their brook trout counterparts ($P < 0.05$). The population census conducted in July 1992 yielded an estimate of 231 brook trout (90% confidence interval of 200–290), 800 brown trout (90% confidence interval of 756–832), and 5 tiger trout, the last comprising approximately 0.5% of the population.

**Table 3.** Mean attributes of male and female trout captured on spawning redds in 1990.

| | | | | | Age | |
|---|---|---|---|---|---|---|
| Trout species | Gender and behavior | *n* | Length (mm) | Weight (g) | Mean (years) | Range (years) |
| Brook | Female, ovulated, digging | 5 | 222±30 | 124±42 | $2.4^{+}\pm0.9$ | 1–3 |
| Brook | Male, subordinate (to brook) | 9 | 188±42 | 127±94 | $1.9^{+}\pm0.6$ | 0–3 |
| Brook | Male, dominant (to brook) | 7 | 238±17 | 154±33 | $2.2^{+}\pm0.8$ | 1–3 |
| Brook | Male, hybridizing, subordinate (to brown) | 7 | 193±28 | 78±29 | $1.6^{+}\pm0.5$ | 0–2 |
| Brown | Female, ovulated digging | 6 | 271±28 | 218±63 | $3.0^{+}\pm0.8$ | 2–4 |
| Brown | Male, subordinate (to brown) | 7 | 235±61 | 184±125 | $2.3^{+}\pm0.8$ | 1–3 |
| Brown | Male, dominant (to brown) | 9 | 345±88 | 476±440 | $4.4^{+}\pm1.6$ | 3–8 |

**Note:** Values are given as the mean ± SD.

## Discussion

The spawning seasons of brook and brown trout in Valley Creek varied little between years and consistently overlapped, during which time behavioral interactions between these species were common. Although direct behavioral interactions between females were not observed, many spawning sites were used by both species with the result that many brook trout redds were almost certainly excavated by later spawning brown trout. Male courtship of heterospecific females was also relatively commonplace, particularly for small brook trout. Although redd superimposition by brown trout has been noted before (Witzel and MacCrimmon 1983), this is the first documented report of hybridization behavior between these species. The frequency of these interactions, the fact that we observed them in all 3 years of this study, and the fact that hybrids are short lived and infertile (Chevassus 1979) leads us to conclude that these species are likely influencing each other's spawning success. Although this conclusion contrasts with that of Witzel and MacCrimmon (1983), the latter study did not perform the systematic long-term behavioral observations of spawning populations of trout that we believe are needed to make this assessment.

At least in Valley Creek, it seems probable that the brook trout is the loser as a result of these interactions. This is because of its smaller relative size, earlier spawning season, and shorter life expectancy. For instance, when evaluating the potential effects of redd superimposition on female reproductive success, it seems highly likely that the earlier spawning brook trout suffers disproportionately because the brown trout not only spawns later, but is also larger and thus likely to dig deeper redds, which would uncover brook trout eggs. In a seemingly analogous situation in Lake Alexandrina (New Zealand), redd superimposition by female rainbow trout was found to reduce the hatching rate of eggs of the earlier spawning brown trout to less than 1% (Hayes 1987).

Exacerbating the situation in Valley Creek is the fact that the fecundity of female brook trout is almost certainly lower than that of its brown trout counterparts because of its smaller size (Bagenal 1978). Similarly, when evaluating the impacts of male hybridization activity, it seems likely that the brook trout again suffers disproportionately because of the relatively greater time male brook trout spends pursuing this activity and the fact that it is relatively short lived compared with male brown trout. A critical factor in this argument is our observation that male hybridization attempts were rarely successful and did not appear to disrupt the spawning activities (success) of female brown trout. Another potentially important factor, and one which we unfortunately cannot evaluate at the present time, is the possibility that the extensive hybridization activity of male brook trout reduces the success of conspecific females simply because it reduces male availability.

Population survey data collected from Valley Creek over the past 35 years lend support to our contention that reproductive interactions between these species may have influenced relative species abundance in this system. Briefly, these data describe a scenario in which brook trout had been living in sympatry with a relatively small but stable population of brown trout for at least 15 (and likely 50) years until the mid-1970s, when the relative abundance of brown trout began to increase concurrent with a decrease in the number of brook trout (Waters 1983). By the late 1970s, the trout population was dominated by brown trout and it has remained this way since (Weigel 1994). Waters (1983) noted a period of flooding in the mid-1960s followed by a siltation event in 1970–1971 and suggested that "innate factors in the behavior of trout, in interaction with habitat perturbations, may have resulted in the replacement of brook trout by brown trout." He suggested that an apparent decrease in watercress (cover for juvenile trout) together with the loss of a year-class may have been the specific factors responsible for the replacement of brook trout. However, this correlation is weak: brook trout abundance actually increased during the first few years following the siltation event, as they had after several earlier floods (Hanson and Waters 1974). Furthermore, although vegetative cover is once more abundant in Valley Creek, brook trout numbers remain low and do not correlate with the presence of cover (Weigel 1994). We suggest that an equally plausible (and perhaps complementary) explanation for the replacement of brook trout is that spawning interactions between these species may have intensified as a result of spawning habitat and (or) redd destruction associated with flooding, that these interactions would have been particularly intense several years after the flood (when any fish born at that time matured), and that once the brown trout came to dominate, spawning interactions would then serve to perpetuate this situation. Interestingly, Waters (1983) noted the presence of tiger trout in Valley Creek in the 1970s. Although we clearly need to know more about factors influencing the reproductive success of individual trout, we believe that there is sufficient reason to hypothesize that interactions between sexually mature brook and brown trout may have played a role in the decline of the brook trout in many North American streams.

The frequency with which brook, brown, and rainbow trout interacted on spawning redds demonstrates that the species-isolating mechanisms between them are not perfect. Both the presence of tiger trout in Valley Creek and our observation of spawning between domesticated brook and brown trout confirmed this phenomenon. Particularly striking was our observation of hybridizing male rainbow trout whose conspecific females do not spawn until March (P.W. Sorensen and T. Essington, personal observations). Ours may be the first account of hybridization behavior between different genera of salmonids. Nonspecific social cues may have promoted nonspecific male reproductive behavior. Pheromones, which have recently been found to play an important role in the reproductive biology of rainbow trout (Scott et al. 1994), and are now known to frequently lack species specificity because of their hormonal origins (Stacey et al. 1994), appear to be candidate stimuli. The discrepancy in size between brook and brown trout may have also influenced these interactions because male salmonids are innately attracted to females larger than themselves, apparently an evolutionary consequence of such females' greater fecundity (Foote and Larkin 1988). Finally, our estimate that less than 1% of Valley Creek trout are hybrids is consistent with our observation that few trout attempting to hybridize succeed, and the fact that the survival rate of hybrid eggs is likely to be extremely low even in hatcheries (Chevassus 1979; Blanc and Chevassus 1986).

Species-isolation mechanisms in salmonids are generally thought to involve three strategies: use of different reproductive behaviors and accompanying social cues, use of different spawning sites, and differences in spawning seasons. Generally, the last is considered to be the most important for naturally sympatric species of salmonids (Heggberget et al. 1988). However, the trout species found in Valley Creek are sympatric as a result of human intervention; there is little reason for them to have evolved species-specific behaviors of any kind. Interestingly, unidirectional hybridization activity between Atlantic salmon, (*Salmo salar*) and brown trout has now been noted (by genetic and biochemical analyses) on both sides of the Atlantic Ocean (McGowan and Davidson 1991; Youngson et al. 1992, 1993). Hybridization activity between recently sympatric salmonid species may be more common and significant than generally supposed.

Although brook and brown trout had similar spawning site preferences, some differences were evident, and spawning site selection appeared to be a relatively specific process. Spawning locations often varied little between years and large areas of the stream were ignored. Beard and Carline (1991) also observed that brown trout used the same sites year to year and hypothesized that they may have a tendency to remain in the vicinity of their natal redd sites. We observed that female brook trout in Valley Creek select spawning areas with upwelling groundwater and relatively low water velocity, while female brown trout dig redds in areas with relatively high water velocities, which appears to confirm previous studies on this topic (Reiser and Wesche 1977; Witzel and MacCrimmon 1983; Grost et al. 1990). Notably, areas used for spawning by both species had intermediate habitat characteristics.

Our analysis of the demographics of trout caught while spawning is the first of its kind and produced several new findings. To our surprise, the age structure of spawning female trout was relatively restricted for both species, strongly suggesting that few female trout survive to spawn in multiple years. The reasons for this are unclear but could be associated with the stress of spawning in this particular stream. However, this trend is not evident in Witzel and MacCrimmon's study (1983). On average, brook trout of both sexes matured at a younger age than brown trout, suggesting that they pursue a different reproductive strategy. A similar but less dramatic trend is also evident in Ontario streams (Witzel and MacCrimmon 1983). The disproportionate abundance of older brown trout males in Valley Creek is an enigma. In both Valley Creek and several Ontario streams (Witzel and MacCrimmon 1983), males of both species tended to mature at a younger age and smaller size than females. Many of our precocious males might have been sneakers, but we lack specific behavioral data on their activity. It would be interesting to examine the age and size structure of an allopatric population of brook trout.

In conclusion, brook and brown trout appear to have similar enough reproductive behaviors and ecologies that when given the opportunity to interact they do. Although the exact significance of these interactions is presently not clear, they probably favor the larger, longer lived brown trout and may explain the replacement of brook trout by brown trout in many systems. Certainly, it seems unlikely that the brook trout, which is the native species, benefits from these interactions. Investigations of the behavioral activities and ecology of individual sexually active brook and brown trout in Valley Creek and elsewhere are now needed to interpret the significance of our findings.

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# The influence of elevation on benthic community responses to heavy metals in Rocky Mountain streams

## William H. Clements and Peter M. Kiffney

**Abstract**: We examined the influence of elevation on benthic macroinvertebrate community responses to heavy metals at 33 sites in six Colorado streams where zinc (Zn) concentration ranged from 2 to 691 μg/L and elevation ranged from 2200 to 3500 m above sea level. We assigned the 33 sites to one of four categories, on the basis of the total Zn concentration and the hardness-based criterion for Zn. Number of taxa, abundance, and species richness of mayflies (Ephemeroptera), and abundance of most mayfly and stonefly (Plecoptera) taxa were significantly reduced at sites where Zn concentration exceeded the hardness-based criterion. Although most measures of benthic community structure were not affected at sites where the Zn concentration was less than the criterion, the species richness of mayflies and the abundance of Heptageniidae, *Drunella*, and *Rhyacophila* were significantly reduced. Results of stepwise multiple regression analysis and canonical discriminant analysis showed that the effects of Zn on benthic community structure were influenced by elevation. These natural changes in benthic communities confound results of biomonitoring studies and must be considered when assessing heavy metal impacts. Total abundance and species richness of mayflies, and abundance of Heptageniidae were the most reliable indicators of heavy metal impacts in our southern Rocky Mountain streams.

**Résumé** : Nous avons examiné l'influence de l'altitude sur les réactions des communautés de macroinvertébrés benthiques aux métaux lourds à 33 sites répartis dans six cours d'eau du Colorado, où la concentration de zinc (Zn) se situait entre 2 et 691 μg/L, et où l'altitude allait de 2200 à 3500 m au-dessus du niveau de la mer. Nous avons classé les 33 sites dans quatre catégories, en fonction de la concentration de Zn total et du critère de dureté établi pour Zn. Le nombre de taxons, l'abondance et la richesse spécifique des éphémères (Ephemeroptera), et l'abondance de la plupart des taxons d'éphémères et de perles (Plecoptera) étaient nettement réduits aux sites où la concentration de Zn dépassait le critère de dureté. Si les mesures de la structure de la communauté benthique n'étaient généralement pas modifiées aux sites où la concentration de Zn était au-dessous du critère, la richesse spécifique des éphémères et l'abondance des Heptageniidae, *Drunella* et *Rhyacophila* étaient nettement réduits. Les résultats d'une analyse de régression multiple séquentielle et d'une analyse discriminatoire canonique ont montré que les effets de Zn sur la structure de la communauté benthique étaient influencés par l'altitude. Ces changements naturels survenus dans les communautés benthiques perturbent les résultats des études de surveillance biologique, et il faut en tenir compte dans l'évaluation des impacts des métaux lourds. L'abondance totale et la richesse spécifique des éphémères ainsi que l'abondance des Heptageniidae étaient les indicateurs les plus fiables des effets des métaux lourds dans nos cours d'eau du sud des Rocheuses.

[Traduit par la Rédaction]

## Introduction

Heavy metal pollution from contemporary and historic mining operations is recognized as one of the most significant environmental problems in western North America (Smith 1987). Since the discovery of gold and other minerals in the Rocky Mountains during the mid-1800s, mining activities have had major impacts on watersheds in this region. In Colorado, heavy metals from over 5000 abandoned mines or tailings piles affect over 2600 km of streams, resulting in the creation of several major hazardous waste sites (Colorado Department of Health 1992).

Benthic invertebrates have been used to monitor effects of heavy metals on streams since the early 1900s (Carpenter 1924). Unlike chemical measures of water quality, benthic communities are directly exposed to varying water quality conditions and therefore integrate effects of contaminants over time (Voshell et al. 1989). Because of their short lifespan, relative immobility, and wide variation in sensitivity

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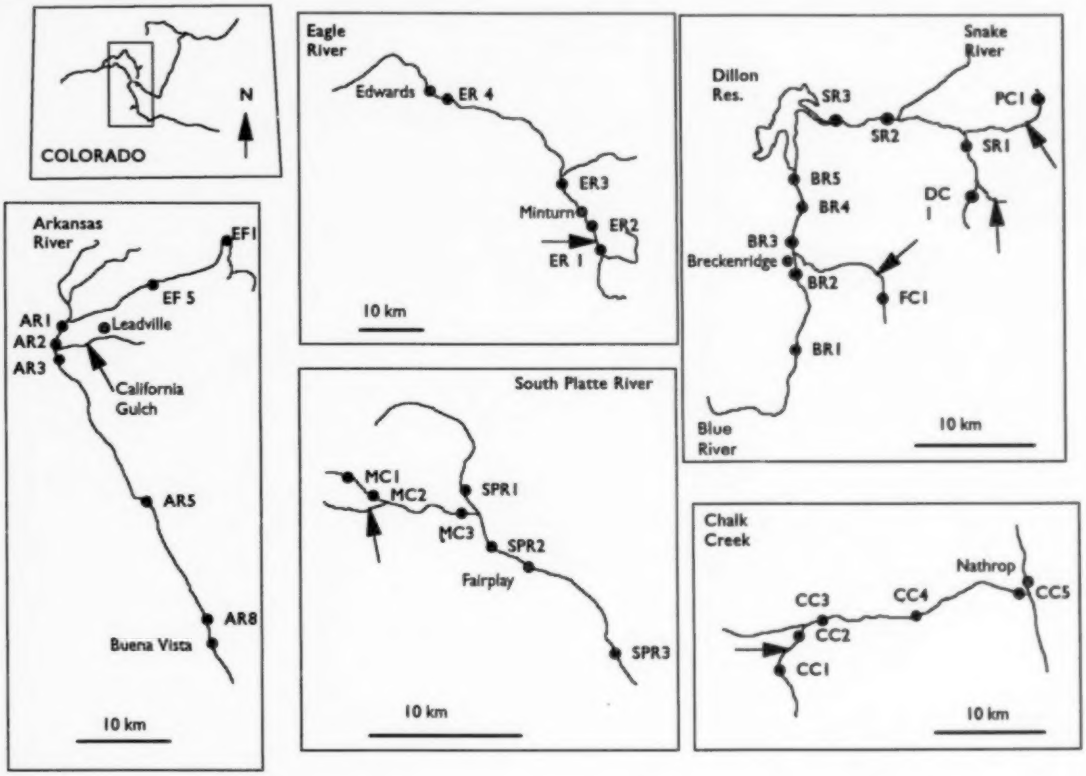
**Fig. 1.** Map of the study area in Colorado showing sampling stations on the Eagle River, Blue River, Snake River, Arkansas River, Chalk Creek, and middle fork of the South Platte River. Arrows indicate approximate locations of known metal inputs.


COLORADO
N
Eagle River
Edwards
ER 4
ER3
Minturn
ER2
ER 1
10 km
Dillon Res.
Snake River
SR3
SR2
PC1
SR1
BR5
BR4
DC 1
BR3
Breckenridge
BR2
FC1
BR1
Blue River
10 km
Arkansas River
EF1
EF 5
AR1
AR2
AR3
Leadville
California Gulch
AR5
AR8
Buena Vista
10 km
South Platte River
MC1
MC2
SPR1
MC3
SPR2
Fairplay
SPR3
10 km
Chalk Creek
Nathrop
CC3
CC4
CC5
CC2
CC1
10 km

to heavy metals, benthic macroinvertebrates are ideal organisms to study effects of mining operations.

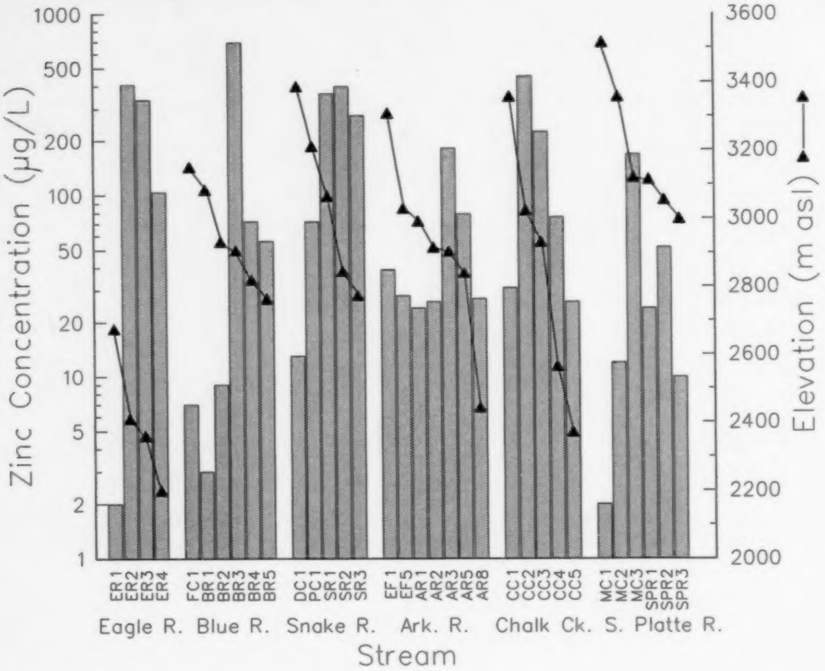
Selecting response variables (end points) to assess effects of contaminants is a critical step when designing biomonitoring studies. While most benthic community measures will respond to severe metal contamination, the choice of appropriate end points is more difficult when evaluating subtle impacts or when measuring recovery in lotic systems. Several recent studies have compared the efficacy of community- and ecosystem-level end points for assessing effects of anthropogenic disturbances (Barbour et al. 1992; Pratt and Bowers 1992; Bruns et al. 1992; Clements 1994). Criteria recommended for evaluating community metrics include sensitivity, sampling variability, uncertainty, redundancy, and cost effectiveness. Kiffney and Clements (1994*b*) also suggested that the performance of benthic community measures should be evaluated experimentally.

Separating natural variation in community structure from variation caused by anthropogenic disturbance is one of the greatest challenges in stream biomonitoring studies. Traditional approaches, in which upstream reference stations are compared with downstream polluted and recovery stations, are confounded by natural changes in community composition (Clements 1994). For example, the river continuum concept (Vannote et al. 1980) predicts longitudinal changes in community structure and function from headwaters to midorder streams and large rivers. In addition, experiments conducted in stream microcosms show that sensitivity to contaminants varies among locations, as benthic communities from small headwater streams are more sensitive to heavy metals than communities from larger rivers (Kiffney and Clements 1994*a*, 1994*b*). We suggest that longitudinal variation in community structure and sensitivity will be especially problematic in high-gradient western streams, where structural and functional changes occur over relatively short distances (Ward 1986; Ward and Stanford 1991).

Assessing effects of heavy metals on stream benthic communities is also limited because of the inability to determine direct cause and effect relationships between metals and community structure. In addition, because most biomonitoring studies are restricted to a single stream, inferences about other metal-affected streams are not statistically valid (Hurlbert 1984). More sophisticated approaches, such as the before after control impact (BACI) design, have been described (Stewart-Oaten et al. 1986, 1992; Smith et al. 1993); however, these approaches are limited to the few situations where pre-impact data are available.

The use of replicated natural experiments to measure the effects of anthropogenic disturbance on community

**Fig. 2.** Total recoverable zinc concentrations (histograms) and elevation (metres above sea level) (triangles) of sampling stations.



structure is rare in stream ecology. Feldman and Connor (1992) used individual replicate streams to assess the effects of pH on macroinvertebrate community structure. They argued that this approach allows researchers to make broader inferences about a larger population of streams. In the present study we used a replicated design to test the hypothesis that heavy metals affected macroinvertebrate community structure in streams receiving mine drainage. Our primary objective was to examine the influence of elevation on benthic community responses to moderate metal concentrations. We also tested the hypothesis that the United States Environmental Protection Agency (U.S. EPA) criterion for zinc (Zn) protected benthic communities. Finally, we make specific recommendations about which measures of benthic community structure are the most reliable indicators of heavy metal pollution in southern Rocky Mountain streams.

## Materials and methods

### Study sites

We sampled 33 stations from six metal-polluted streams in central Colorado in August 1992 (Fig. 1, Table 1). Stations (four to seven per stream) were selected on the basis of elevation and distance from known metal inputs (Fig. 2). The Blue River, Snake River, and Eagle River are located on the west side of the continental divide, while the Arkansas River, middle fork of the South Platte River, and Chalk Creek are located on the east side.

Four stations were sampled in the Eagle River, located between Red Cliff and Eagle, Colo. The reference station (ER1) was located upstream from the Eagle Mine, a U.S. EPA Superfund site, and the remaining three stations were located downstream from this site. Five stations were sampled in the Snake River drainage system, including stations in Deer Creek (DC1) and Peru Creek (PC1), the two major tributaries to the Snake River. The Snake River and Peru Creek receive discharge from numerous mines located near Montezuma, Colo. Six stations were sampled in the Blue River drainage system, near Breckenridge, Colo. FC1 was a reference station on French Creek, 7.2 km upstream from the Blue River. French Creek flows through extensive placer mine tailings downstream from FC1 and is the primary source of metals to the Blue River. Stations BR1 and BR2 were located on the Blue River upstream from the confluence with French Creek, and stations BR3, BR4, and BR5 were located downstream from this confluence. Seven stations were sampled in the Arkansas River, upstream and downstream from California Gulch, a U.S. EPA Superfund site near Leadville, Colo. Stations EF1 and EF5 were located in the East Fork of the Arkansas River, upstream from Leadville, Colo. Stations AR1 and AR2 were located upstream from California Gulch, and stations AR3, AR5, and AR8 were located downstream. We sampled five stations in Chalk Creek, a tributary to the Arkansas River located 15 km downstream from Buena Vista, Colo. Although CC1 was initially considered a reference station, metals analysis showed elevated levels of copper (Cu) and Zn at this station (see Results). Stations CC2, CC3, CC4, and CC5 were located downstream from known metal inputs. We sampled six stations in the middle

**Table 1.** Physical and chemical characteristics of sampling stations at the Eagle River, Blue River, Snake River, Arkansas River, Chalk Creek, and middle fork of the South Platte River, Colorado.

| Station | Depth (cm) | Width (m) | Canopy (%) | Current velocity (cm/s) | pH | Dissolved oxygen (mg/L) | Conductivity (μS/m) | Temperature (°C) | Alkalinity (mg/L) | Hardness (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Eagle River | | | | | | | | | | |
| ER1 | 25.3 | 6.2 | 5.5 | 64.2 | 8.5 | 8.4 | 180 | 11 | 119 | 122 |
| ER2 | 19.0 | 15.5 | 5.2 | 78.5 | 8.0 | 8.0 | 145 | 14 | 52 | 84 |
| ER3 | 39.3 | 21.1 | 13.1 | 64.0 | 8.0 | 8.0 | 150 | 15 | 54 | 86 |
| ER4 | 43.0 | 22.0 | 5.0 | 117.9 | 8.8 | 7.8 | 240 | 18 | 77 | 126 |
| Blue River | | | | | | | | | | |
| FC1 | 14.3 | 4.7 | 30.0 | 47.5 | 7.8 | 8.6 | 55 | 9 | 30 | 42 |
| BR1 | 27.0 | 7.2 | 15.0 | 113.9 | 7.5 | 8.2 | 75 | 11 | 43 | 50 |
| BR2 | 31.0 | 7.3 | 45.0 | 61.8 | 8.1 | 8.1 | 85 | 12 | 52 | 60 |
| BR3 | 39.3 | 14.3 | 14.0 | 70.9 | 7.9 | 8.3 | 100 | 12 | 42 | 66 |
| BR4 | 30.0 | 17.0 | 7.5 | 99.0 | 7.3 | 8.3 | 105 | 11 | 52 | 64 |
| BR5 | 51.7 | 10.9 | 6.6 | 79.4 | 7.8 | 8.4 | 100 | 9 | 51 | 64 |
| Snake River | | | | | | | | | | |
| DC1 | 13.7 | 2.8 | 31.0 | 52.3 | 7.5 | 7.6 | 65 | 10 | 10 | 10 |
| PC1 | 10.0 | 1.5 | 25.0 | 54.7 | 7.3 | 8.1 | 70 | 8 | 17 | 48 |
| SR1 | 28.0 | 4.5 | 17.0 | 76.1 | 6.7 | 7.5 | 100 | 11 | 11 | 52 |
| SR2 | 37.0 | 10.5 | 0.0 | 62.4 | 6.8 | 7.6 | 90 | 14 | 16 | 48 |
| SR3 | 34.7 | 11.9 | 9.0 | 72.3 | 6.8 | 7.3 | 90 | 16 | 18 | 48 |
| Arkansas River | | | | | | | | | | |
| EF1 | 19.3 | 8.7 | 18.8 | 62.5 | 7.8 | 7.4 | 90 | 12 | 32 | 48 |
| EF5 | 28.0 | 5.1 | 10.0 | 65.2 | 8.4 | 8.8 | 170 | 8 | 70 | 108 |
| AR1 | 32.7 | 13.3 | 8.9 | 64.9 | 8.2 | 7.6 | 155 | 13 | 64 | 94 |
| AR2 | 30.3 | 6.9 | 17.4 | 65.6 | 8.0 | 7.6 | 150 | 15 | 67 | 92 |
| AR3 | 24.7 | 10.7 | 2.0 | 92.7 | 7.9 | 7.6 | 170 | 15 | 69 | 100 |
| AR5 | 45.0 | 12.6 | 4.4 | 84.0 | 8.8 | 8.2 | 170 | 17 | 65 | 98 |
| AR8 | 38.0 | 20.1 | 0.0 | 96.3 | 7.8 | 7.3 | 100 | 18 | 34 | 44 |
| Chalk Creek | | | | | | | | | | |
| CC1 | 10.0 | 1.5 | 80.0 | 53.9 | 8.2 | 8.0 | 55 | 8 | 23 | 32 |
| CC2 | 20.7 | 9.5 | 36.4 | 73.8 | 7.3 | 7.8 | 75 | 10 | 23 | 40 |
| CC3 | 24.0 | 12.1 | 10.9 | 50.5 | 7.3 | 7.6 | 75 | 11 | 28 | 36 |
| CC4 | 46.0 | 9.0 | 50.0 | 49.5 | 7.4 | 7.6 | 80 | 15 | 31 | 42 |
| CC5 | 29.3 | 7.3 | 30.2 | 81.7 | 7.9 | 6.8 | 140 | 21 | 50 | 52 |
| South Platte River | | | | | | | | | | |
| MC1 | 20.3 | 4.8 | 18.8 | 65.3 | 7.8 | 7.6 | 40 | 11 | 17 | 18 |
| MC2 | 15.0 | 2.0 | 10.0 | 55.9 | 7.9 | 7.3 | 75 | 14 | 35 | 40 |
| MC3 | 32.3 | 4.5 | 24.1 | 52.5 | 8.3 | 7.2 | 155 | 16 | 69 | 96 |
| SPR1 | 31.3 | 5.5 | 18.7 | 60.8 | 8.5 | 7.5 | 130 | 16 | 64 | 80 |
| SPR2 | 35.0 | 13.0 | 7.5 | 82.4 | 8.7 | 7.3 | 150 | 16 | 62 | 86 |
| SPR3 | 32.7 | 10.8 | 8.5 | 49.7 | 8.2 | 6.8 | 210 | 20 | 89 | 110 |

fork of the South Platte River (SPR) drainage near Fairplay, Colo. Three stations were located in Mosquito Creek, a tributary to the SPR and the primary source of metals to this system. Stations MC1 and MC2 were located upstream and station MC3 was located downstream from metal inputs. Station SPR1 was located upstream from the confluence with Mosquito Creek, and stations SPR2 and SPR3 were located downstream from this confluence.

### Water quality

Stream temperature, pH, conductivity, and dissolved oxygen content were measured at all stations when benthic macroinverebrates were collected. Water samples were collected from all stations and hardness, alkalinity, and heavy metal concentrations were analyzed in the laboratory using standard procedures (U.S. Environmental Protection Agency 1983). To determine total recoverable metals, samples (14 mL) were acidified with reagent-grade nitric acid to a pH of less than 2.0. Samples for dissolved metals were filtered through a 0.45-μm filter and acidified. Concentrations of cadmium (Cd), Cu, and Zn were determined using flame or furnace atomic absorption spectrophotometry. Quality assurance, quality control, and percent recovery were determined by analyzing known standards and acid blanks.

**Table 2.** Mean hardness-based Zn criterion values and Zn concentrations (total recoverable) measured at background, low-, medium-, and high-Zn stations.

| Zn category | Stations included | Mean Zn criterion (μg/L) | Mean Zn concentration (μg/L) |
|---|---|---|---|
| Background (<0.25*X*)[a] | FC1, BR1, BR2, MC1, MC2, ER1, SPR1, SPR3, EF5, AR1, AR2 | 82.1 | 13.3 |
| Low (0.25–1.0*X*) | CC5, EF1, SPR2, ER4, DC1, AR5, AR8, BR5 | 76.4 | 49.5 |
| Medium (1.1–2.0*X*) | AR3, BR4, MC3, CC1, CC4, PC1 | 72.4 | 100.0 |
| High (>2.0*X*) | BR3, CC2, CC3, SR1, SR2, SR3, ER2, ER3 | 66.9 | 393.6 |

[a] *X* = hardness based criterion for Zn.

### Benthic invertebrates

Benthic invertebrate samples ($n = 5$) were collected using a 0.1-m$^2$ Hess sampler from shallow (<0.5 m) riffle areas at each station. Substrate was disturbed to a depth of approximately 10 cm and materials were washed into a 350-μm mesh net. Samples were sieved (350 μm) in the field and organisms retained were preserved in 70% ethanol. In the laboratory, samples were sorted and organisms were identified to the lowest practical taxonomic level (genus or species for most taxa; subfamily or tribe for chironomids).

### Statistical analyses

All statistical analyses were performed using a PC version of the Statistical Analysis System (SAS Institute Inc. 1988). Each of the 33 stations was assigned to one of four categories (background, low, medium, and high Zn), on the basis of the total recoverable Zn concentration and the U.S. EPA hardness-based water quality criterion for Zn at that station (see Results). The water-quality criterion for Zn was determined using the following equation: criterion value = $e^{(0.847\ \ln(\text{hardness})+0.860)}$ (U.S. Environmental Protection Agency 1986). For example, a station with a water hardness of 50 mg/L would have a Zn criterion value of 64.9 μg/L. We defined background, low-, medium-, and high-Zn stations as sites where Zn concentration was less than 0.25, 0.25–1.0, 1.1–2.0, and greater than 2.0 times the criterion value, respectively. We used one-way analysis of variance (ANOVA) to test for differences in total abundance; number of taxa; abundance and species richness of mayflies (Ephemeroptera); and abundance of the 13 dominant taxa among Zn categories. Dominant taxa were defined as those groups that comprised >1% of the benthic community in any Zn category. All analyses were performed using log-transformed variables because inspection of residual plots showed heterogeneity of variances. If differences were significant ($p < 0.05$), Ryan's *Q* multiple range test was used to test for differences among Zn categories. Ryan's *Q* is a conservative test that has been recommended because of its rigorous control of experimentwise error rates (Day and Quinn 1989).

We examined separation of stations in each Zn category using canonical discriminant analysis (Dillon and Goldstein 1984), based on abundance of the 13 dominant taxa. Contributions of each dominant taxon to separation of stations were based on the relative magnitude of loading coefficients. Stepwise multiple regression (forward selection) was used to examine the relationship between benthic community measures and each abiotic variable measured (elevation, zinc concentration, pH, hardness, alkalinity, conductivity, temperature, dissolved oxygen content, current velocity, depth, and width).

## Results

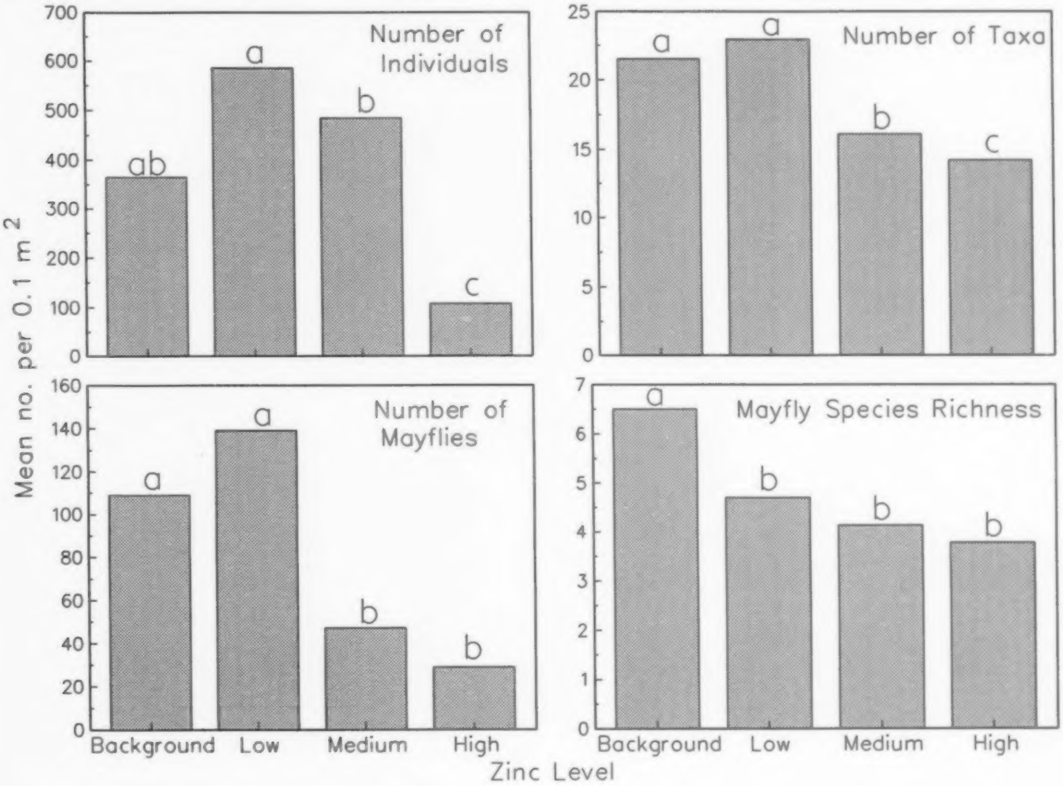
### Physical and chemical characteristics

Elevation of the 33 stations ranged from 2195 to 3511 m above sea level (asl) (Fig. 2); however, most stations were located in the upper montane and subalpine zones (2450–3350 m asl) (Ward 1986). Temperature, stream width, depth, conductivity, alkalinity, and hardness increased from upstream to downstream in all streams (Table 1) and were inversely correlated with elevation (temperature, $r = -0.54$, $p = 0.0013$; stream width, $r = -0.76$, $p = 0.0001$; depth, $r = -0.60$, $p = 0.0002$; conductivity, $r = -0.52$, $p = 0.0018$; alkalinity, $r = -0.36$, $p = 0.0387$; hardness, $r = -0.41$, $p = 0.0163$). Levels of dissolved oxygen and pH showed little variation among stations. Substrate at all stations was dominated by cobble (64–250 mm diameter), with varying amounts of gravel and boulder. Canopy cover was greatest in smaller, headwater streams and consisted primarily of willow (*Salix* sp.).

Zn was the predominant heavy metal measured at all stations in all streams. Because levels of Cd and Cu were almost always below detection, only Zn concentrations are presented here. The only exception was station CC1, where total recoverable Cu was 44 μg/L. Zn concentrations are reported as total recoverable metals because differences between total and dissolved concentrations during the low flow conditions when this study was conducted were negligible (usually <10%).

Zn concentrations ranged from 2 μg/L at upstream reference stations ER1 and MC1 to 691 μg/L at Blue River station BR3, immediately downstream from Mosquito Creek (Fig. 2). Zn levels exceeded the hardness-based criterion value at all stations immediately downstream from known metal inputs and decreased with distance

**Fig. 3.** Mean total number of benthic invertebrates, number of taxa, number of mayflies, and species richness of mayflies per 0.1 $m^2$ at background, low-, medium-, and high-Zn stations. Zn concentrations at these stations were <0.25, 0.25–1.0, 1.1–2.0, and >2.0 times the U.S. EPA hardness-based criterion, respectively. Means with the same letter were not significantly different ($p < 0.05$).



downstream. An exception to this pattern was the Snake River, where Zn levels remained elevated at all downstream stations.

Mean hardness-based criterion values, mean Zn concentrations, and stations included in the four Zn categories are shown in Table 2. We included station CC1 as a medium-Zn station because both Zn and Cu concentrations were elevated (31 and 44 μg/L, respectively). The mean hardness-based criterion value for Zn ranged from 66.9 to 82.1 μg/L within each category. Mean Zn concentrations within categories ranged from 13.3 μg/L at background stations to 393.6 μg/L at high-Zn stations. The mean Zn concentration in the medium-Zn category was moderately elevated (100.0 μg/L) and exceeded the mean hardness-based criterion value (72.4 μg/L).

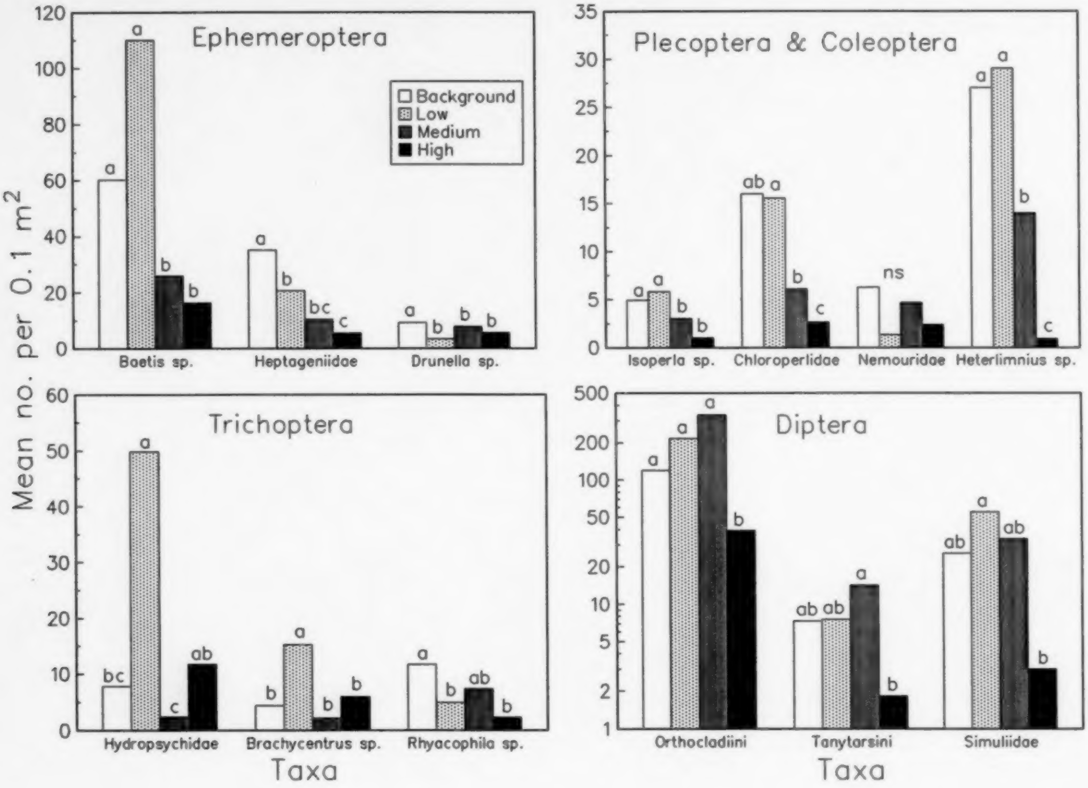
### Benthic invertebrates

Total abundance, number of taxa, number of mayflies, and species richness of mayflies varied significantly among Zn levels, but the relationship between these variables and Zn was complex (Fig. 3). Mean number of individuals increased by 60% from background to low-Zn sites; however, this increase was not statistically significant because of large variation among stations within these Zn categories. The number of individuals was significantly reduced only at the high-Zn sites. The mean number of taxa was similar at background and low-Zn sites, but decreased significantly at medium-Zn sites. The total number of mayflies was also significantly reduced at the medium- and high-Zn sites. Species richness of mayflies was the only community-level variable significantly reduced at low-Zn sites compared with background sites.

Thirteen dominant macroinvertebrate taxa accounted for 87–93% of the total individuals within any Zn category. Abundance of all groups except nemourid stoneflies (Plecoptera) varied significantly among Zn categories (Fig. 4). Abundance of baetid mayflies (*Baetis bicaudatus* and *Baetis tricaudatus*) was highly variable at background and low-Zn stations and was significantly reduced only at medium- and high-Zn stations. Abundance of Heptageniidae (*Rhithrogena hageni*, *Epeorus longimanus*, *Epeorus deceptivus*, and *Cinygmula* sp.) and *Drunella* sp. was significantly reduced at low-, medium-, and high-Zn stations.

Abundances of *Isoperla* sp. and Chloroperlidae (Plecoptera) and *Heterlimnius corpulentus* (Coleoptera: Elmidae) were similar at background and low-Zn stations but

**Fig. 4.** Mean abundance of the 13 dominant taxa per 0.1 m$^2$ at background, low-, medium-, and high-Zn stations (see caption of Fig. 3 for details).



were significantly reduced at medium- and high-Zn sites. Similarly, abundance of the predatory caddisfly *Rhyacophila* sp. (Trichoptera) was greatest at background sites and lowest at the high-Zn stations. In contrast, abundance of Hydropsychidae (*Hydropsyche oslari*, *Hydropsyche cockerelli*, and *Arctopsyche grandis*) and Brachycentridae, two groups of filter-feeding caddisflies, was low at background, medium-, and high-Zn stations, but significantly elevated at the low-Zn stations.

Orthoclad chironomids (Diptera) showed an apparent threefold increase in abundance from background to medium-Zn sites; however, this apparent increase was not significant because of variation among stations within these Zn categories. Orthocladiinae dominated the benthic community at most stations downstream from metal inputs and accounted for 70% of the total individuals at medium-Zn sites. Abundance patterns for filter-feeding black flies (Simuliidae) were similar to those for Hydropsychidae and Brachycentridae. Black fly abundance was significantly reduced only at high-Zn sites.

## Multivariate analyses

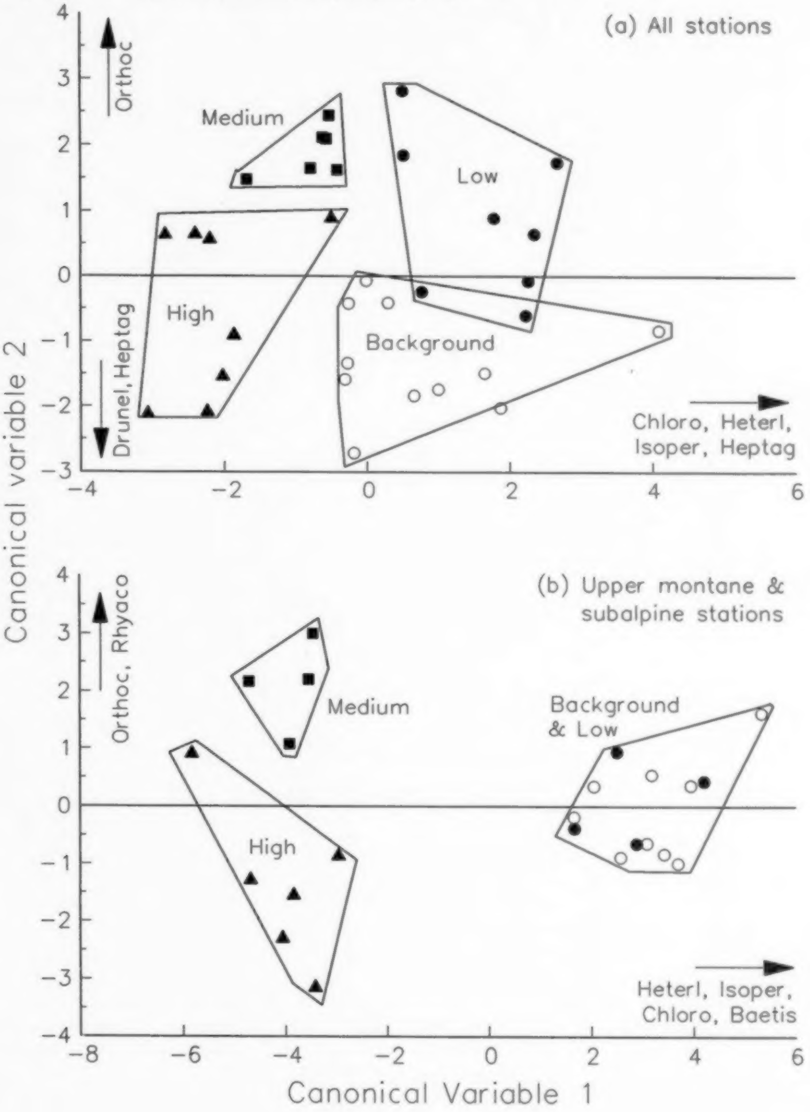
Separation of background, low-, medium-, and high-Zn sites was evaluated using canonical discriminant analysis. The first analysis included all 33 stations and showed moderate separation among Zn categories (Fig. 5*a*). Background and low-Zn sites were distinct and separated from medium- and high-Zn stations along canonical variable 1 because of greater abundance of Chloroperlidae, *Heterlimnius*, *Isoperla*, and Heptageniidae. Separation along canonical variable 2 resulted from greater abundance of Orthocladiinae and lower abundance of *Drunella* and Heptageniidae at low- and medium-Zn sites.

We conducted a second canonical discriminant analysis to examine the influence of elevation on separation of stations in the four Zn categories. This analysis included only stations located in the upper montane and subalpine zones ($n = 23$) (Fig. 5*b*). Although background and low-Zn sites overlapped broadly when alpine and lower montane stations were removed, we observed much greater separation of the other Zn categories. Separation along canonical variable 1 resulted from greater abundance of *Heterlimnius*, *Isoperla*, Chloroperlidae, and *Baetis* at background and (or) low-Zn stations. Separation of medium- from high-Zn stations along canonical variable 2 resulted from higher abundance of Orthocladiinae and *Rhyacophila* at medium-Zn stations.

## Stepwise multiple regression analyses

Results of stepwise multiple regression analysis showed that Zn concentration, elevation, and several other variables

**Fig. 5.** Canonical discriminant analyses showing overlap and separation of background, low-, medium-, and high-Zn stations when all stations were included (*a*) and when the analysis was restricted to the 23 stations in the upper montane and subalpine zones (*b*). Taxa responsible for separation along each canonical axis and the direction that these taxa separate stations (indicated by arrows) are provided. Baetis, *Baetis*; Chloro, Chloroperlidae; Drunel, *Drunella*; Heptag, Heptageniidae; Heterl, *Heterlimnius*; Isoper, *Isoperla;* Orthoc, Orthocladiinae; Rhyaco, *Rhyacophila*.



significantly correlated with elevation (i.e., temperature, width, depth, conductivity) explained a significant amount of variation in benthic community structure (Table 3). Overall $r^2$ values ranged from 0.18 for Tanytarsini to 0.70 for Brachycentridae. Although significant regression models were obtained for most groups, Zn was not included as a predictor variable for several dominant taxa. Abundance of filter-feeding caddisflies and black flies (Hydropsychidae, Brachycentridae, Simuliidae) was strongly associated with conductivity and (or) stream width. Similarly, abundance of the mayfly *Drunella* sp. was influenced primarily by temperature and alkalinity. Zn concentration was a significant predictor for 8 of the 17 variables and explained the greatest variation in total Ephemeroptera ($r^2 = 0.26$), and abundance

**Table 3.** Stepwise multiple regression (using forward selection) showing the relationship between benthic community measures, total recoverable Zn concentration, and other abiotic measures.

| Benthic measure | Overall $F$ | Overall $r^2$ | Variables included in model (partial $r^2$) |
|---|---|---|---|
| Individuals | 10.6*** | 0.52 | (+)pH (0.40), (+)current (0.06), (−)Zn (0.06) |
| Taxa | 10.0*** | 0.59 | (+)pH (0.36), (−)Zn (0.10), (+)width (0.06), (−)temperature (0.07) |
| Ephemeroptera richness | 6.6** | 0.31 | (+)dissolved $O_2$ (0.18), (−)Zn (0.13) |
| Ephemeroptera abundance | 10.1** | 0.40 | (−)Zn (0.26), (−)elevation (0.14) |
| *Baetis* sp. | 14.4*** | 0.49 | (−)elevation (0.26), (−)Zn (0.23) |
| Heptageniidae | 10.7*** | 0.53 | (−)current (0.24), (−)Zn (0.21), (−)temperature (0.08) |
| *Drunella* sp. | 6.1** | 0.53 | (−)temperature (0.23), (−)alkalinity (0.10), (−)conductivity (0.08), (+)width (0.06), (−)depth (0.06) |
| *Isoperla* sp. | 19.8*** | 0.39 | (+)alkalinity (0.39) |
| Chloroperlidae | 17.1** | 0.36 | (−)Zn (0.36) |
| Nemouridae | 13.6*** | 0.48 | (−)conductivity (0.37), (−)depth (0.11) |
| Hydropsychidae | 14.2*** | 0.49 | (+)width (0.38), (+)conductivity (0.11) |
| Brachycentridae | 16.6*** | 0.70 | (+)conductivity (0.51), (+)temperature (0.10), (+)dissolved $O_2$ (0.05), (−)hardness (0.04) |
| Rhyacophilidae | 11.5*** | 0.50 | (+)dissolved $O_2$ (0.26), (−)conductivity (0.12), (+)alkalinity (0.07), (−)depth (0.05) |
| *Heterlimnius* sp. | 9.9*** | 0.51 | (+)alkalinity (0.30), (−)Zn (0.12), (−)temperature (0.09) |
| Orthocladiinae | 10.3*** | 0.40 | (+)pH (0.29), (+)current (0.11) |
| Tanytarsini | 3.3 (ns) | 0.18 | (+)dissolved $O_2$ (0.18) |
| Simuliidae | 8.9** | 0.22 | (+)conductivity (0.22) |

**Note:** The table shows overall $F$ values and $r^2$ values for the complete model, significant partial $r^2$ values for each abiotic variable included in the model, and the directional effect (+,−) of the abiotic variable on each benthic community measure. ns, not significant; **, $p < 0.01$; ***, $p < 0.001$.

of Chloroperlidae ($r^2 = 0.36$), Heptageniidae ($r^2 = 0.21$), and *Baetis* sp. ($r^2 = 0.23$).

## Discussion

Our results show that benthic invertebrate communities in Colorado streams were altered at sites where Zn concentration exceeded the U.S. EPA water-quality criterion. Specifically, the number of taxa, abundance, and species richness of mayflies, and the abundance of most mayfly and stonefly taxa were significantly reduced at medium-Zn stations compared with background stations. Medium- and high-Zn stations were dominated by metal-tolerant orthoclad chironomids and (or) hydropsychid caddisflies (Winner et al. 1980; Petersen and Petersen 1983; Yasuno et al. 1985; Clements et al. 1988), a finding that is consistent with patterns observed in other western streams (Peckarsky and Cook 1981; Chadwick et al. 1986; Roline 1988; Leland et al. 1989; Moore et al. 1991; Clements 1994) and measured experimentally in stream microcosms (Clements et al. 1992; Kiffney and Clements 1994*a*, 1994*b*). Because this study was not restricted to a single stream, our results have broader implications for a larger population of metal-polluted streams in Colorado. Although we could not randomly assign metal "treatments" to stations, our study design is superior to that employed in traditional biomonitoring studies, in which upstream reference stations are compared with downstream polluted stations within the same stream. Changes in benthic community structure reported here may represent a general pattern in western streams polluted by mining.

Most measures of benthic community structure were not significantly different between background and low-Zn categories. Results of canonical discriminant analysis also showed considerable overlap between these categories, particularly when the analysis was restricted to upper montane and subalpine stations. Because Zn concentrations at low-Zn stations were less than the criterion value but greater than background levels, our results suggest that the U.S. EPA water quality criterion for Zn protected most benthic organisms included in this study.

Some measures of benthic community structure, such as species richness of mayflies and abundance of Heptageniidae, *Drunella*, and *Rhyacophila*, were significantly reduced at low-Zn stations compared with background sites. While the sensitivity of mayflies to heavy metals is well documented (Specht et al. 1984; Clements et al. 1988, 1992), especially among species in the family Heptageniidae

(Nelson and Roline 1993; Clements 1994; Kiffney and Clements 1994*b*), reduced abundance of these groups at low-Zn stations may not have resulted entirely from Zn exposure. Stepwise multiple regression analysis showed that Zn was an important predictor of heptageniid abundance; however, it was not an important predictor for *Drunella* or *Rhyacophila*. In fact, previous studies have shown that *Rhyacophila* is highly tolerant of heavy metals and often abundant in metal-contaminated streams (Leland et al. 1989; Clements 1994). Effects of heavy metals on *Drunella* in experimental streams are dependent on instar, with large instars being relatively tolerant to metals (Kiffney and Clements 1994*b*). Finally, canonical discriminant analysis showed that separation of background and low-Zn stations resulted from greater abundance of *Drunella* and Heptageniidae at background stations when all stations were included in the analysis. However, when this analysis was restricted to stations at similar elevations these taxa were not important and background and low-Zn stations overlapped broadly. These results suggest that differences in abundance of Heptageniidae, *Drunella*, and *Rhyacophila* between background and low-Zn stations were confounded by elevation and (or) stream size. Background sites were generally located at higher elevations than low-Zn sites (2670–3511 vs. 2195–3304 m asl, respectively). In addition, stream width and depth were less at background sites than at low-Zn sites (Table 1). Because of these habitat differences, we cannot attribute the reduced abundance of Heptageniidae, *Drunella*, and *Rhyacophila* observed at low-Zn stations exclusively to Zn.

In contrast, reduced species richness of mayflies at low-Zn stations may have resulted from elevated Zn levels. Ward's idealized species richness curves for mountain streams predict increased species richness of mayflies from headwaters to midorder streams (Ward 1986). Despite higher elevation and smaller stream size, species richness of mayflies was greatest at background sites. These results imply that species richness of mayflies may be a sensitive indicator of low-level metal pollution in southern Rocky Mountain streams.

Abundance patterns for *Isoperla*, Chloroperlidae, and *Heterlimnius* were similar and suggested that these groups were sensitive to moderate Zn concentrations. Canonical discriminant analysis showed that these taxa were also important for separating background and low-Zn stations from medium- and high-Zn stations. Zn was the only variable included in the multiple regression model for Chloroperlidae and was an important predictor of abundance of *Heterlimnius*. These results suggest that reduced abundances of Chloroperlidae, *Isoperla*, and *Heterlimnius* may be useful indicators of moderate metal pollution in southern Rocky Mountain streams.

Variation in the abundance of filter-feeding caddisflies (Hydropsychidae, Brachycentridae) among background, low-, and medium-Zn stations was probably not related to heavy metals. Although we obtained highly significant multiple regression models for Hydropsychidae and Brachycentridae, Zn was not an important predictor in either model. Abundance of both groups was significantly correlated with stream width, conductivity, or other variables associated with elevation, a finding that is consistent with other research reporting low abundance in headwater streams (Ward 1986). Previous field studies (Winner et al. 1980; Clements et al. 1988; Clements 1994) and microcosm experiments (Kiffney and Clements 1994*b*) have shown that caddisflies are tolerant of heavy metals. We conclude that filter-feeding caddisflies were able to tolerate Zn and that their distribution was probably related to other factors, such as stream size and elevation.

Abundance patterns for the three dominant groups of dipterans were generally similar. Orthoclad chironomids dominated the benthic community at most stations downstream from metal inputs and were significantly reduced only at high-Zn stations. The tolerance of orthoclads for heavy metals is well documented, and the increased abundance of this group is considered to be a typical response to heavy metal pollution (Winner et al. 1980; Clements et al. 1988; Waterhouse and Farrell 1985; Clements 1994; Kiffney and Clements 1994*b*).

## Conclusions

Although most measures of benthic community structure were affected at stations with the highest Zn concentrations (i.e., >2.0 times the U.S. EPA criterion), distinguishing metal effects at moderately polluted stations was more difficult and required consideration of natural longitudinal changes in community structure. Abundance and species richness of many invertebrates in western streams increase naturally from upstream to downstream (Ward 1986; Ward and Stanford 1991). These natural changes in benthic communities, which occur over relatively short distances in western streams, confound results of biomonitoring studies and may limit our ability to detect significant differences between reference and moderately polluted sites.

Natural variability in benthic community structure must be considered when selecting community measures to assess heavy metal impacts, especially in moderately polluted systems. We recommend the following criteria to select community measures of metal pollution in Rocky Mountain streams. First, the measure should be significantly affected at low to moderate metal concentrations. In the present study we defined moderate Zn levels as 1.1–2.0 times the U.S. EPA criterion. The mean Zn concentration at our medium-Zn stations (100.0 μg/L) exceeded the hardness-based criterion value for Zn (72.4 μg/L). Second, the measure should be significantly associated with Zn concentration. Several measures of benthic community structure reported in this study meet these two criteria, including number of taxa; species richness and abundance of mayflies; and abundance of *Baetis*, Heptageniidae, Chloroperliidae, and *Heterlimnius*.

Because biomonitoring studies cannot demonstrate direct cause and effect relationships between changes in benthic community structure and heavy metals, measures should be evaluated experimentally. In experiments conducted in stream microcosms, we have shown that number of taxa; total abundance and species richness of Ephemeroptera; and abundance of *Baetis* and Heptageniidae are highly sensitive to heavy metals (Kiffney and Clements 1994*b*). Although number of taxa is often a useful indicator of metal impacts in streams, replacement of sensitive taxa by tolerant taxa may complicate this measure (Clements

1994). The usefulness of *Baetis* as an indicator of metals pollution in Rocky Mountain streams is questionable because of its potential for rapid recolonization from upstream or downstream sources (Peckarsky and Cook 1981; Hershey et al. 1993; Clements 1994). Microcosm experiments show that some species in the family Chloroperlidae (i.e., *Sweltsa coloradensis*) are relatively tolerant to moderate metal levels (Kiffney and Clements 1994*b*), which may limit the usefulness of this group. Finally, there is little experimental data on the sensitivity of *Heterlimnius* to heavy metals. On the basis of these considerations, we conclude that the total abundance and species richness of mayflies and the abundance of Heptageniidae (*Rhithrogena hageni*, *Epeorus longimanus*, *Epeorus deceptivus*, and *Cinygmula* sp.) are the most reliable indicators of metal pollution in southern Rocky Mountain streams.

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# Application of a one-dimensional model to vertical distributions of cod eggs on the northeastern Newfoundland Shelf

**John T. Anderson and Brad deYoung**

**Abstract**: A one-dimensional model is developed to describe the vertical distribution of cod eggs and larvae on the northeastern Newfoundland Shelf. The model is dependent on egg buoyancy, temperature-dependent development, and age-dependent changes in density for eggs in good and poor condition. The model was fit to physical oceanographic data from the inner and outer shelf collected in 1991. Output from the model compared favourably with field observations. Cod eggs (stages I to III) were bimodally distributed above and below 100 m depth. Late stage eggs (stage IV) and larvae (5–6 mm) were only abundant in surface waters (<50 m). There was a progression from deeper to shallower depths as cod eggs developed through to larvae. Cod eggs observed deeper in the water column were thought to be in poor condition and were probably sinking. The model demonstrates that the vertical distribution of cod eggs is sensitive to changes in water temperature, water density (salinity), and egg condition.

**Résumé** : Nous avons élaboré un modèle unidimensionnel pour décrire la distribution verticale des oeufs et des larves de morue sur la partie nord-est du plateau continental de Terre-Neuve. Le modèle est tributaire de la flottabilité des oeufs, du développement dépendant de la température et des changements dépendants de l'âge dans la densité des oeufs en bonne et en mauvaise condition. Le modèle a été ajusté avec les données d'océanographie physique recueillies en 1991 dans la partie inférieure et extérieure du plateau. Le résultat du modèle se comparait favorablement aux observations sur place. Les oeufs de morue (stades I–III) présentaient une distribution bimodale au-dessus et au-dessous d'une profondeur de 100 m. Les stades plus tardifs (stade IV) et les larves (5–6 mm) abondaient seulement dans les eaux superficielles (<50 m). On note une progression des profondeurs vers les eaux moins profondes au fil du développement des oeufs et jusqu'aux larves. Les oeufs de morue observés en profondeur dans la colonne d'eau semblaient en mauvaise condition et étaient probablement en train de s'enfoncer. Le modèle démontre que la distribution verticale des oeufs de morue est sensible aux changements de la température de l'eau, de sa densité (salinité) et de la condition des oeufs.

[Traduit par la Rédaction]

## Introduction

Egg and larval drift of Northern cod is reported to occur over vast geographic distances and is thought to be an important phase in their life history (Templeman 1981; Lear and Green 1984). Spawning in spring occurs at considerable depths (300–600 m) in relatively warm waters (2–4°C) along the Newfoundland and Labrador shelf edge and in the deep waters overlying the shelf (Templeman 1966, 1981; May 1966). Spawning also takes place across the shelf (Hutchings et al. 1993) and to a limited extent in the bays (Templeman 1981; Wroblewski et al. 1994). Previously it has been assumed that fertilized eggs rise quickly to surface waters where they develop and hatch into larvae. Because of cold temperatures and the strong advective flow of the Labrador Current, downstream drift has been predicted to play a significant role in the early life history of Northern cod (Templeman 1981; Lear and Green 1984; Helbig et al. 1992). Studies are currently underway to model the dispersion of cod eggs in relation to spawning concentrations on the northeastern Newfoundland Shelf. The vertical distributions of fish eggs and larvae is an important component in describing the drift of eggs and larvae in the presence of strong current shear (Page et al. 1989; Sundby 1991). When eggs rise slowly to surface waters, downstream transport may be significantly reduced. When eggs hatch at depths inappropriate for larval feeding then survival may be reduced (Page and Frank 1989; Page et al. 1989).

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Previous studies on cod eggs have demonstrated that egg density changes during development. In Norwegian and Baltic cod, eggs reportedly increase in density during development (Sundnes et al. 1965; Mangor-Jensen 1987; Nissling and Westin 1991). These eggs are thought to be positively buoyant throughout development and have been classified as pelagic, with concentrations increasing exponentially towards the surface when the water column is unstratified (Sundby 1983, 1991; Westgård 1989). Ontogenetic changes in egg density are not, however, always consistent among species (Page et al. 1989). Anderson and deYoung (1994), for example, demonstrated that cod eggs from Newfoundland waters decrease in density during development. In contrast, egg density from Norwegian cod increased during development (Sundnes et al. 1965; Mangor-Jensen 1987; Nissling and Westin 1991). Differences in density can occur as a result of egg quality, with eggs being more dense when quality is poorer (Sundnes et al. 1965; Kjesbu et al. 1992). In addition, cod egg density can increase during development when eggs develop in poor water conditions (Anderson and deYoung 1994).

The purpose of this study was to model the vertical distribution of cod eggs and larvae from the northeastern Newfoundland Shelf. First, we modify previously developed models of egg concentration profiles (Sundby 1983; Page et al. 1989) to apply to the Newfoundland area where we have experimentally measured the density of cod eggs and where the waters are neither vertically mixed nor linearly stratified. Second, we examine the consequences of varying cod egg density as a function of egg development and egg quality on the modelled vertical distributions. Direct measurements of egg density on the shelf are used as input to the model. Model predictions are then compared with field observations.

## Methods

### Egg concentration model

The one-dimensional model that we develop has the same basis as the earlier work of Sundby (1983) and Page et al. (1989), a simplified form of the one-dimensional advection–diffusion equation (Gill 1982), with the horizontal advection terms removed.

$$[1] \qquad \frac{\partial C}{\partial t} + \frac{\partial (WC)}{\partial z} = \frac{\partial}{\partial z}\left(K_v \frac{\partial C}{\partial z}\right) - MC$$

where $C$ is the concentration, $W$ is the vertical egg velocity, $K_v$ is the vertical eddy diffusivity, and $M$ is mortality. Page et al. (1989) argued that the mortality term scales as a small term in this equation and can be ignored. In particular, the mortality term can be ignored if there is no temporal or vertical bias because it will not affect the form of the steady-state vertical distribution, although it will of course influence the total number of eggs present. With this assumption, then the steady-state version of eq. 1 becomes

$$[2] \qquad \frac{\partial}{\partial z}\left(WC - K_v \frac{\partial C}{\partial z}\right) = 0$$

Throughout this discussion we shall consider the vertical diffusivity to be constant, independent of depth. We do not follow Page et al. (1989). Rather, we choose $K_v = 10^{-2}\ \mathrm{m^2 \cdot s^{-1}}$, a high value considered to be reasonable for the southwestern Scotian Shelf (Loder et al. 1988). However, we shall instead set $K_v = 10^{-3}\ \mathrm{m^2 \cdot s^{-1}}$, a value found by Petrie et al. (1991) to be applicable to the inner northeastern Newfoundland Shelf on the basis of an analysis of salinity data there. Although theoretical (Gargett 1984), observational (Stigebrandt 1976; deYoung and Pond 1988), and modelling studies (Mathieu and deYoung 1994; Umoh and Thompson 1994) indicate that vertical diffusivity can vary with depth, we shall assume a constant vertical diffusivity in the absence of a clear model to apply to the northeastern Newfoundland Shelf. Determination of the spatial and temporal variability of the vertical structure of $K_v$ is an important but unresolved problem in oceanography.

The solution to eq. 2 is found by assuming that the concentration is zero at great depth, a reasonable assumption for the analysis that we apply here. The resulting solution for $C(z)$ with $K_v$ constant is

$$[3] \qquad C(z) = \frac{C_0}{K_v}\exp\left(-\int_z^0 W\,\mathrm{d}z\right)$$

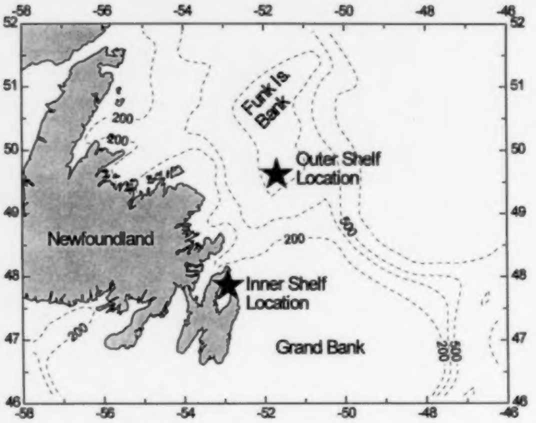
where $C_0$ is the egg concentration at the surface ($z = 0$). This equation has been applied by both Sundby (1983) and Page et al. (1989). The two unknowns in this equation are the vertical diffusivity ($K_v$) and the vertical egg velocity ($W$), which depends on the egg density, water density, egg diameter, and the viscosity of water. Sundby (1983) ignored the effects of water column stratification entirely while Page and Frank (1989) assumed linear stratification for numerical simplicity. We use the observed density profiles, and solve all the equations numerically, making no simplifying assumptions from this point onwards.

To solve eq. 3 we must obtain the vertical egg velocity profile. Previous work attempting to compare the descent trajectories of cod eggs through a density gradient column with that predicted by theory indicated that cod eggs did not descend as predicted (Anderson and deYoung 1994). In addition, these experiments also indicated that the descent profiles of glass beads were predicted poorly by theory. In this paper, we pursue the question of applying the most appropriate mathematical formulation to the velocity profile of a cod egg through water.

The terminal velocity of an egg is a general problem in fluid dynamics that depends upon the egg and water densities, the viscosity of water, and the size of the egg. The form of the applicable solution depends upon a dimensionless number, the Reynolds number, that is defined as $\mathrm{R_e} = \rho_w W d/\mu$, where $\rho_w$ is the water density, $W$ is the vertical egg velocity, $d$ is the object diameter, and $\mu$ is the dynamic viscosity of the fluid. At high Reynolds number, $\mathrm{R_e} \gg 1$, inertial terms dominate and viscosity is unimportant. At low Reynolds numbers, $\mathrm{R_e} \ll 1$, viscosity is important and inertial terms are unimportant. For the rising eggs, the $\mathrm{R_e}$ is approximately 1, whereas for the beads that are used to calibrate the egg densities, $\mathrm{R_e} \leq 50$. Thus, for eggs both viscosity and inertia are important, whereas beads are clearly in the inertial regime (Kundu 1990).

We present three different formulations for the vertical velocity function, for application to both the egg and bead laboratory data. The data were collected as part of laboratory measurements of cod egg density, where cod eggs (or glass

**Fig. 1.** Study area showing the inner and outer shelf areas sampled in 1991, for the vertical distribution of cod eggs. Depth contours are in metres.



beads) are introduced into the top of a linear density gradient, as described by Anderson and deYoung (1994). These models are necessary for the application to the model of egg ascent and distribution in the solution of eq. 3. The first formulation is Stokes' law (Kundu 1990), which applies for $R_e < 0.5$.

$$[4] \qquad W = \frac{d^2 g(\rho_w - \rho_e)}{18\nu}$$

where $d$ is the egg diameter (mm), $g$ is the gravitational constant (9.81 $m \cdot s^{-2}$), $\rho_e$ is the egg density ($kg \cdot m^{-3}$), and $\nu$ is the molecular viscosity of sea water ($1.61 \times 10^{-3}$ $kg \cdot m^{-1} \cdot s^{-1}$ at 3°C and 35 ppt).

The second equation, attributed to Allen (1900), was used by Sundby (1983) and Page et al. (1989) where it was referred to as Dallavalle's equation (Dallavalle 1947).

$$[5] \qquad W = \frac{K_i d_0 (\rho_w - \rho_e)^{2/3}}{\nu^{1/3}}$$

In this equation $K_i$ is a constant determined from observations by Allen (1900) as 0.0882 $m^{5/3} \cdot kg^{-1/3} \cdot s^{-4/3}$, $\rho_w$ is water density ($kg \cdot m^{-3}$). The particle diameter $d$ is replaced by an effective diameter $d_0$ where $d_0 = d - 0.4D$. In this equation, $D$ is the diameter defined by Allen (1900) as

$$[6] \qquad D = \sqrt[3]{\frac{36\nu^2}{2g\,|\rho_w - \rho_e|\,\rho_w}}$$

In applying Allen's equation we use Stokes' law for small Reynolds numbers because $D$ blows up as $(\rho_w - \rho_e) \rightarrow 0$.

The third formulation that we will consider for the terminal velocity is attributed to Gibbs et al. (1971) who derived a result for particles in the size range 0.000 63 cm < $d$ < 1.0 cm.

$$[7] \qquad W = \frac{-3\nu + \sqrt{9\nu^2 + gd^2(s - 1)l_1(d)}}{l_2(d)}$$

where $s = \rho_e/\rho_w$, $l_1(d) = 0.003\,869$ cm $+ 0.02480d$, and $l_2(d) = 0.011\,607$ cm $+ 0.07440d$. Only the last two equations should apply to the bead data, for which the Reynolds number is high, but in theory all three of these equations should apply to the egg data, for $R_e < 1$.

## Egg trajectory model

An egg trajectory model was formulated as a subset of the egg concentration model to examine biological effects on vertical distributions of cod eggs over the Newfoundland Shelf. We wanted to measure the effects of ontogenetically varying egg density, owing to variable egg quality, and slow egg development rates, owing to passage through the cold intermediate layer (<0°C), on hypothetical egg trajectories through the water column.

The model is based on eq. 5 but differs in its treatment of egg density ($\rho_e$), which is written as

$$[8] \qquad \rho_e = \alpha_0 + \beta A_e$$

where $\alpha_0$ is egg density at day zero ($kg \cdot m^{-3}$), $\beta$ represents the rate of change in egg density from fertilization to hatch ($kg \cdot m^{-3} \cdot d^{-1}$), and $A_e$ is egg age (d). The initial density was set to 1026.5 $kg \cdot m^{-3}$, as determined for eggs artificially fertilized at the time of capture from cod captured at 380 m depth on the Grand Bank (Anderson and deYoung 1994). $\beta$ was allowed to vary from $-0.22$ $kg \cdot m^{-3} \cdot d^{-1}$ for eggs in the best condition to 0.07 $kg \cdot m^{-3} \cdot d^{-1}$ for eggs in poor condition (Anderson and deYoung 1994). Egg age is estimated as a function of temperature and is used to scale the rate of change of egg density measured in the laboratory at 3°C, such that for each time step in the model,

$$[9] \qquad A_e = A_e + \frac{20}{H}t$$

where $H$ is time to hatch (d) and $t$ is time (d). The laboratory measurements of egg density were made at 3°C where total development time is ~20 d. The modelled ontogenetic changes in egg densities were scaled to fall within the range of laboratory values according to temperature-dependent development times for cod eggs, as determined by Apstein (1909, after Page and Frank 1989),

$$[10] \qquad H = a(T + 2)^b$$

where $T$ is the observed water temperature (°C) and $a$ = 74.131 and $b = -0.82$. Model runs demonstrated that low temperatures (less than $-1$°C) experienced by cod eggs passing through the water column resulted in egg development times >>100 d on the basis of Apstein (1909), a result of the near asymptotic fit at low temperatures. Therefore, the maximum development time was constrained to be ≤50 d, although these times may in fact be longer. Temperature and water density were based on measured values from the field program conducted in 1991 at inner and outer shelf locations (Fig. 1). The time step for each model run was ≤1 d.

## Observed vertical egg distributions

Sampling to determine the vertical distributions of cod eggs and larvae on the northeastern Newfoundland Shelf was carried out during late June and early July 1991 (Fig. 1). At each site repeated tows were made every 3 h using a multiple opening and closing sampling system that is similar in design and operation to the BIONESS (Sameoto et al. 1977). On the inner shelf, sampling was conducted over a 48-h period and on the outer shelf over a 24-h period. The sampler was fit with nine 0.333-mm mesh nets, each with a mouth area of 0.25 m$^2$. Opening and closing of each net was controlled in real time from the ship. The sampler was fitted with a variety of sensors that allowed precise sampling close to the bottom and throughout the water column in relation to the physical water structure and plankton biomass. During each tow the BIONESS sampler was lowered to within 1 m of the bottom and the first net was opened. Sampling of each depth stratum was uniform, where a minimum of 80 m$^3$ of water was filtered. Depth strata were chosen such that shallower depths were sampled with a greater resolution. For example, depth strata in the upper 50 m of the water column ranged between 10 and 30 m, whereas deeper depth strata ranged from 50 to 60 m. The deepest stratum sampled ranged from 30 to 45 m. Temperature and salinity data were collected by performing a Seabird-19 conductivity–temperature–depth profile prior to each BIONESS tow.

Plankton samples were preserved in 5% buffered Formalin and processed in the laboratory. Eggs were classified following the four stage criteria outlined by Markle and Frost (1985) while yolk sac larvae were designated as stage V. Early cod eggs cannot be distinguished from haddock (*Melanogrammus aeglifinus*) or witch flounder (*Glyptocephalus cynoglossus*) eggs (Fahey 1983); however, haddock do not occur on the northeastern Newfoundland Shelf and witch flounder larvae were not encountered in our samples. We assume that all eggs were cod. Center of mass distributions were estimated for each stage as a function of depth in the water column by $D = \Sigma_{i=1}^{n} (p_i d_i)$, where $D$ is the center of mass (m), $p_i$ is the proportion of eggs or larvae sampled in the $i$th stratum, and $d_i$ is the mean depth of the $i$th stratum.

## Results

### Formulation of vertical velocities

Glass beads of known density were used to calibrate the density gradient column (Anderson and deYoung 1994). Although they are larger (4–7 mm) than the eggs (1.2–1.6 mm), they provide a useful test of the velocity equations discussed above. The position or height of the beads in the density gradient column was observed every 5–10 s. We fit a spline curve to the height data and then differentiated the spline to obtain the vertical descent rate of the beads. Results from a typical bead descent are plotted in Fig. 2*a*, as well as the velocity curves attributed to Stokes', Allen's, and Gibbs' equations. The Reynolds number for the falling beads ranged from 0 to 50; hence, one would not expect Stokes' law to hold over most of this range and it does not. The Gibbs' curve, which becomes asymptotic with Stokes' curve at low Reynolds number, does not fit the data well but is an improvement on Stokes' law. Allen's equation provides a good fit to the observations.

The egg data produced a somewhat more equivocal result (Fig. 2*b*). For most of the descent profile the cod eggs descended through the density gradient column at considerably lower velocities than predicted by theory. The data show that neither Stokes' nor Gibbs' equations provided good fits to the observations, while Allen's equation only fit the data well at the lowest velocities. The Reynolds number for the falling eggs ranged from zero to one. Over most of the profile shown in Fig. 2*b*, $R_e \simeq 0.5$, suggesting that Allen's law should apply. These results demonstrate that Allen's equation provides the best fit to the observed descent velocity for these eggs, of the three formulations.

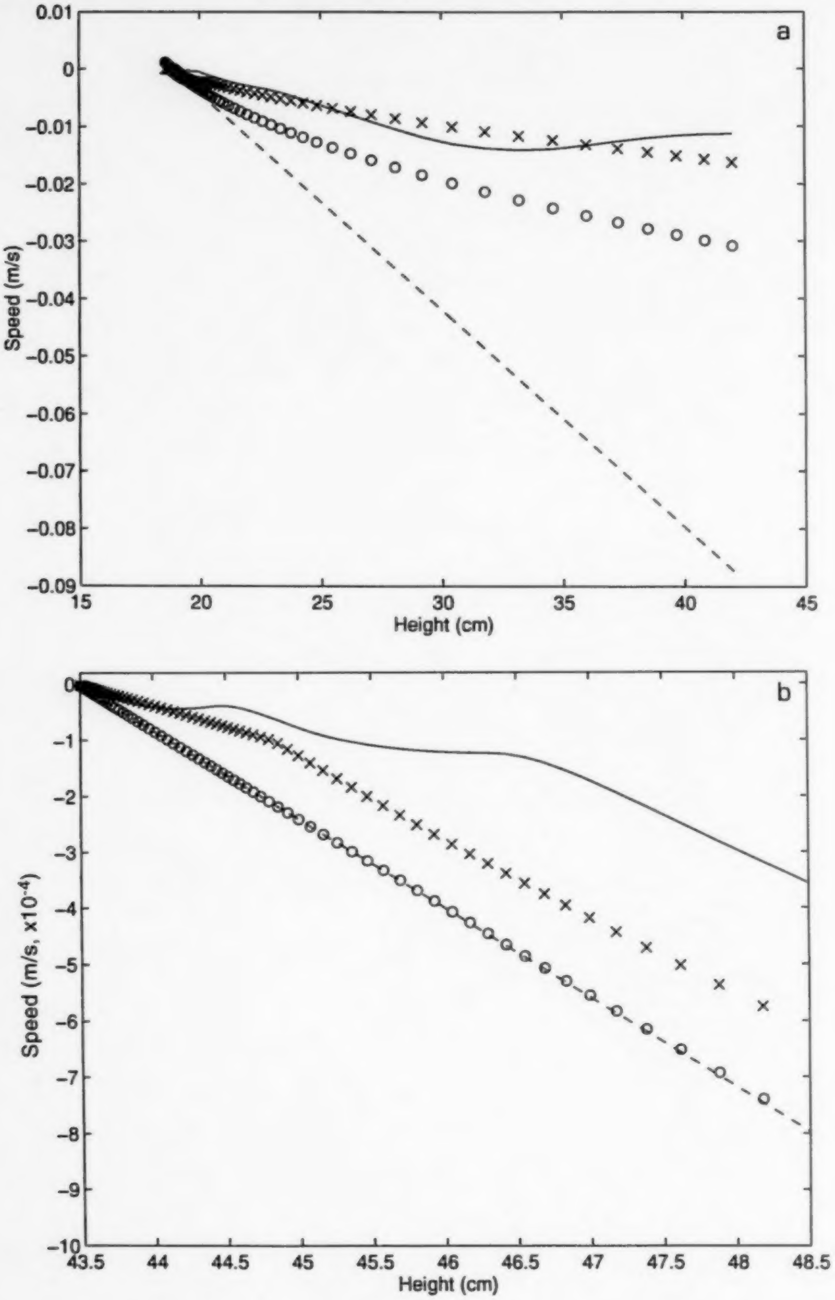
Note that this analysis is based upon the assumption that the particles being tracked have reached terminal velocity. For low Reynolds number, less than one, the terminal velocity of the eggs is reached in roughly 0.1 s (from $2/9\, d^2 \rho_w / \mu$; Kundu 1990). The maximum egg velocities recorded were approximately 0.0004 m·s$^{-1}$, although most of the egg descents occurred at velocities <0.0001 m·s$^{-1}$. Therefore, the eggs typically fell <0.000 01 m in the time necessary to reach terminal velocity, and not more than 0.000 04 m at maximum descent velocities. We think it is a reasonable assumption that the eggs reached terminal velocities in the density gradient column. The beads, which are in a higher Reynolds number region, roughly 50, take approximately 1 s to reach terminal velocity, during which time they would travel approximately 0.01 m at the maximum velocities observed. It is thus possible that the beads have not reached terminal velocity at the start of their descent, when the largest deviations tended to occur. However, this does not appear to be the explanation for the somewhat poorer fit of the egg velocity profiles, which may result from osmotic regulation by the eggs (Anderson and deYoung 1994).

Another question concerns the sensitivity of the model results to the choice of velocity formulation, either Stokes' law or Allen's equation. Page et al. (1989) estimated that the use of Stokes' law leads to an error of 25%. A plot of the velocities for the two different formulations (calculated from the outer shelf density data shown in Fig. 4*b*) suggests that the error is much higher, as high as 50% (Fig. 3). Use of Stokes' equation would significantly overestimate cod egg ascent times. The resulting concentration profile calculated for an arbitrary egg density of 1022.5 kg·m$^{-3}$ applied to eq. 3 also shows that there can be significant sensitivity to the profile of vertical velocity (see below).

### Modelled egg concentrations

The temperature and density profiles averaged for the inner and outer Newfoundland shelf areas were noticeably different. On the outer shelf the temperatures were relatively warm at 300 m depth, decreased from approximately 3°C to minima of −0.9°C at 60 m depth and then increased again to approximately 3°C at the surface (Fig. 4*a*). On the inner shelf temperatures were less than −1°C for water >50 m depth to the bottom at 280 m. Above 50 m, water temperatures warmed rapidly to surface values of approximately 5.5°C. Water density profiles indicated that waters >50 m depth were relatively well mixed for both the inner and outer shelf areas (Fig. 4*b*). Above 50 m, densities decreased to minima at the surface. The sharper density gradient observed at 50 m depth for the inner shelf resulted from averaging water densities measured at many stations, where the density structure varied among samples. At all

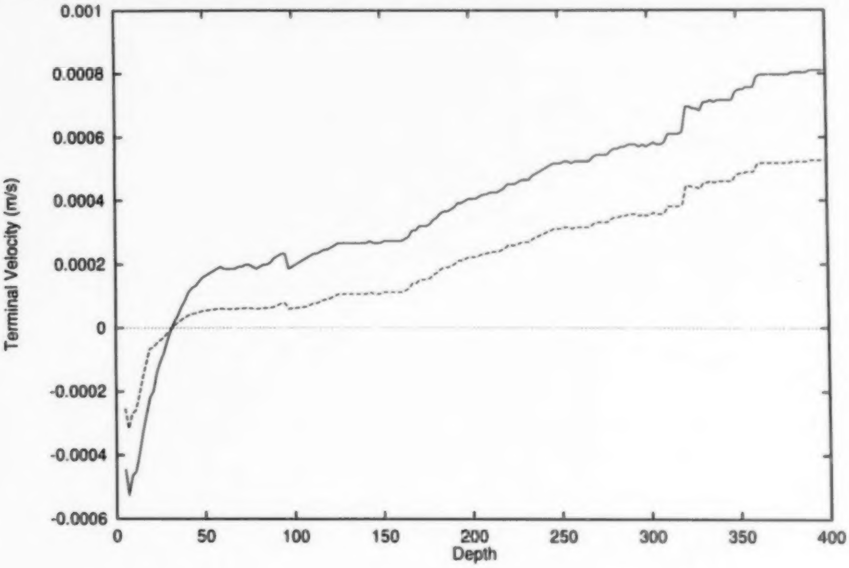
**Fig. 2.** Descent velocities for (*a*) a single glass bead (solid line) and (*b*) a single cod egg (solid line) in the density gradient column. Simulated velocities are shown for Stokes' law (broken line), Allen's equation (×) and Gibbs' equation (○).



depths the water density was notably greater for the outer shelf stations.

As an indication of the relative importance of the water density profile, we show the vertical concentrations of eggs found for water density data from the outer Newfoundland Shelf using a linear density gradient and the observed density gradient. Initial conditions for the model set egg density at 1026.5 $kg{\cdot}m^{-3}$, as measured in the laboratory for

**Fig. 3.** Simulated vertical velocity profile for a cod egg using Stokes' law (solid line) and Allen's equation (broken line) for the same water density profile.



cod eggs from the outer shelf (Anderson and deYoung 1994). Water density at this location was 1027.34 kg·m$^{-3}$ at 300 m depth (Anderson and deYoung 1994) such that initial egg buoyancy ($\Delta\rho = \rho_w - \rho_e$) was equal to 0.84 kg·m$^{-3}$. Egg density was allowed to decrease at a rate of −0.22 kg·m$^{-3}$·d$^{-1}$ for a 20-d period. Shown is the steady state solution for 20 d. For a linearly stratified water column, cod eggs rose much more slowly because of a lower buoyancy throughout the subsurface waters (Fig. 5). The only advantage of the linear gradient is analytical simplicity (Page and Frank 1989). However, the analytical solution, chosen for simplicity, will lead to significant differences from the numerical solution.

To compare the effect of geographical differences in water density structure, we modelled the egg concentration profiles for each day during development from fertilization to hatch. This differs from Page et al. (1989) who modelled four discrete egg stages, and Sundby (1983, 1991) and Westgård (1989) who modelled all stages together. This version of the model (eq. 3) ignores temperature effects on egg development time. In this regard the modelled time steps are relative as the true hatching time for cod eggs on the northeastern Newfoundland Shelf will be >20 d (see below). Therefore, time step 8 simply represents the point when eggs hatch. Initial conditions were set as described above, where initial egg density was set at 1026.5 kg·m$^{-3}$. This resulted in a lower initial buoyancy on the inner shelf ($\Delta\rho = 0.20$), where water densities at 280 m were lower.

Modelled egg concentration profiles for the inner and outer shelf areas differed, where eggs on the inner shelf took approximately twice as long to reach surface waters (<50 m depth). On the inner shelf, all cod eggs rose above 50 m depth by time step 5 with the highest concentrations being ~25 m depth (Fig. 6*a*). On the outer shelf, where

**Fig. 4.**(*a*) Temperature (°C) profiles averaged for all stations sampled offshore (dot–dash line) and inshore (broken line) in 1991. (*b*) Water density ($\sigma_t$) profiles averaged for all stations sampled offshore (dot-dash line) and inshore (broken line) in 1991.

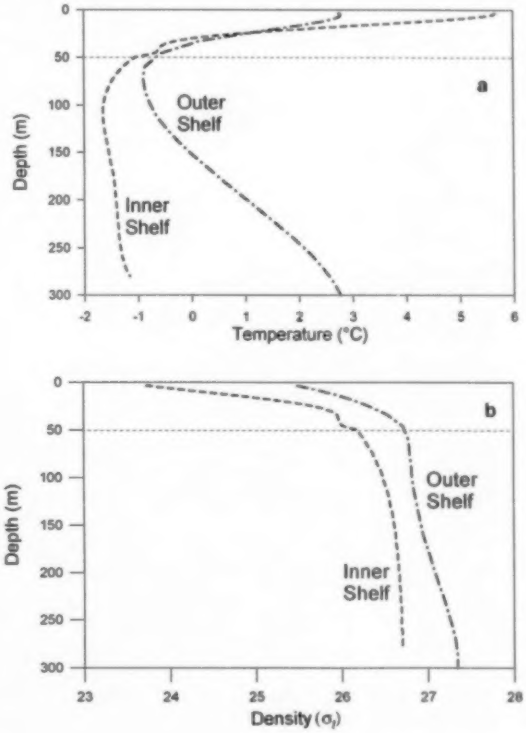
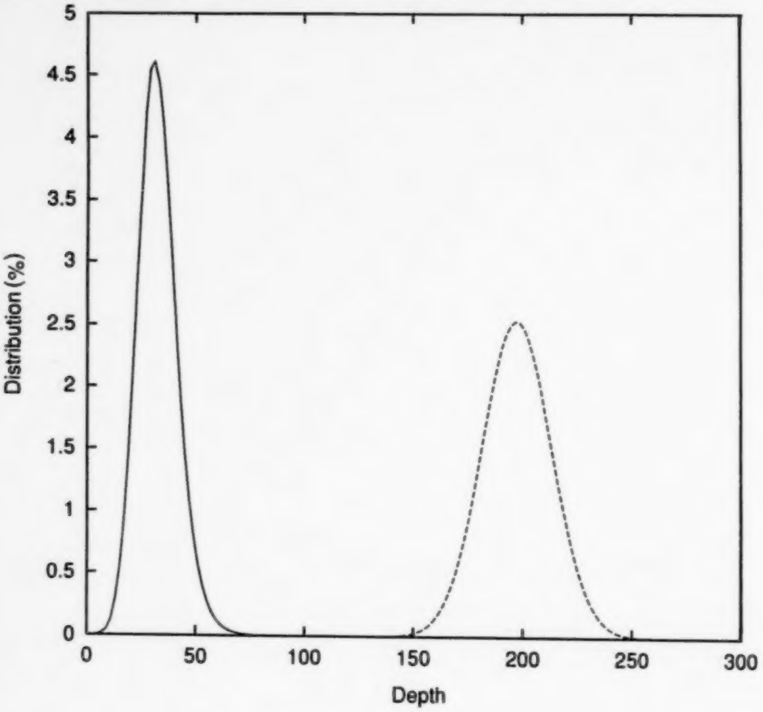


**Fig. 5.** Plot of the vertical distribution of an arbitrary concentration of eggs in a linear density gradient (broken line) and a real density gradient (solid line). The linear density gradient was obtained by interpolating between the surface and the bottom values of the real density gradient. Allen's equation was used to generate the vertical velocity.



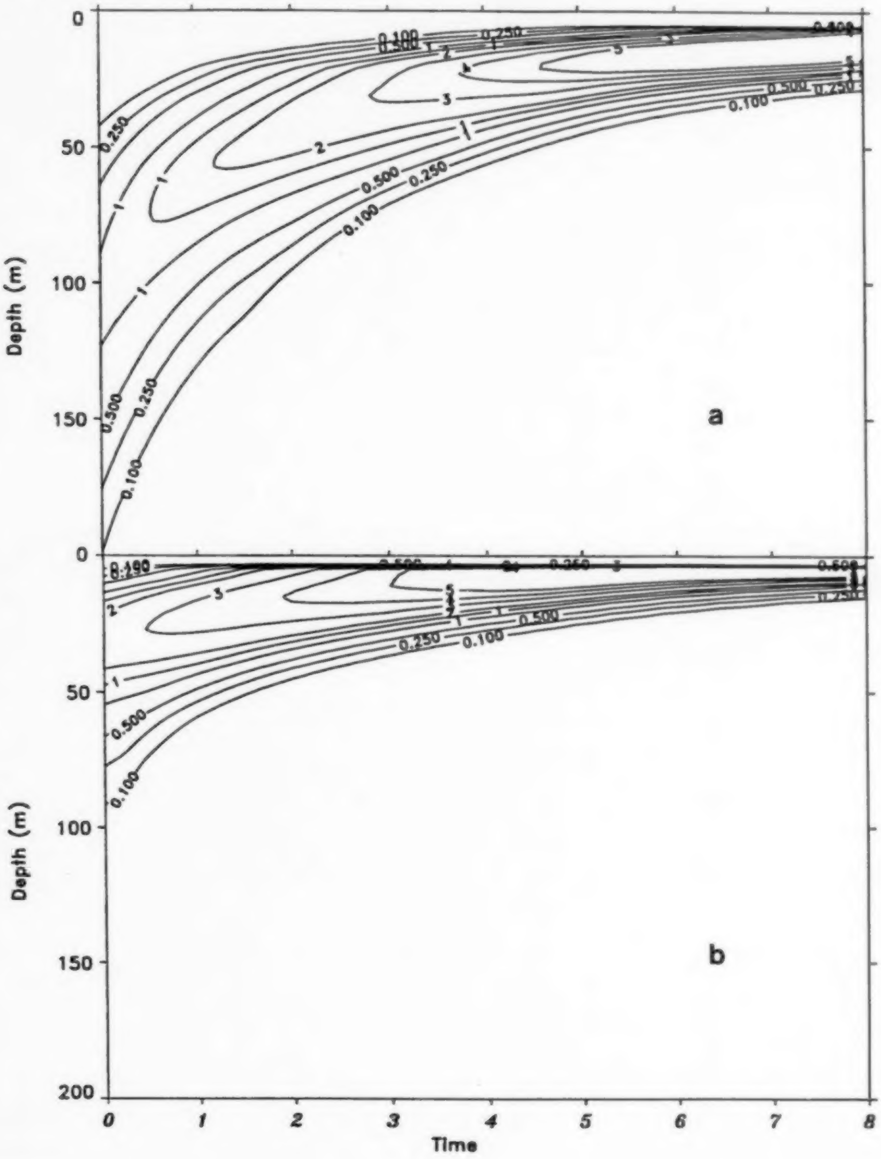
the water density at a given depth was greater, cod eggs rose much more quickly to surface waters with all of the eggs being <50 m depth by time step 2 while the highest concentrations were again ~25 m depth (Fig. 6*b*). At the point of hatch all eggs were <30 m depth on the inner shelf and <20 m depth on the outer shelf. The difference in egg concentration profiles between the inner and outer shelf results, in part, from a difference in initial buoyancy of 0.64 $kg{\cdot}m^{-3}$. However, this difference is relatively small compared with the modelled rate of change in egg density of $-0.22\ kg{\cdot}m^{-3}{\cdot}d^{-1}$.

### Egg trajectory model

Good egg condition was defined by a high initial buoyancy and high rate of decrease in egg density during development (Anderson and deYoung 1994). For poor egg condition, we allowed the initial buoyancy to remain high but the rate of change in egg density was allowed to vary from negative to positive, on the basis of laboratory measurements (Anderson and deYoung 1994). Modelled time–depth trajectories for eggs in good condition demonstrated that eggs would rise rapidly from 300 m depth to surface waters, reaching 50 m depth in approximately 7 d and the surface in 10 d on the outer shelf (Fig. 7). On the inner shelf eggs rose from 280 m depth and reached 50 m depth in approximately 10 d and the surface waters at 21 d of age (Fig. 7). The slower rate of ascent for the inner shelf is a function of lower water density coupled with colder subsurface water temperatures, which prolong development and slow the rate of decrease in egg density. The 50 m depth is referenced as the depth above which cod eggs would be exposed to greater surface current velocities and wind mixing (Petrie et al. 1991). This model predicts that cod eggs would hatch at approximately 24 d of age on the outer shelf and 27 d on the inner shelf, on the basis of the observed temperature and salinity structure in June 1991.

When the rate of change in egg density during development is allowed to vary between estimates for eggs in good condition ($-0.22\ kg{\cdot}m^{-3}{\cdot}d^{-1}$) and eggs in poor condition ($0.07\ kg{\cdot}m^{-3}{\cdot}d^{-1}$) we generate a family of ascent trajectories (Fig. 8). These results demonstrate that egg condition and the vertical structure of water density determine where a cod egg might hatch. On the outer shelf, cod eggs that do not change density throughout development become neutrally buoyant at ~30 m depth (Fig. 8). When specific gravity increases during development cod eggs on the outer shelf would settle deeper in the water column, below the mixed-layer depth, with eggs actually sinking in the water column (Fig. 8). Repeating this procedure for the inner shelf area demonstrated a greater effect on cod egg trajectories, where cod eggs ended up deeper in the water column because of the lower buoyancies in less dense waters.

**Fig. 6.** Egg concentration profiles modelled for (*a*) inner and (*b*) outer shelf areas for a relative time scale from fertilization to hatch, derived from the observed water density structures measured in 1991 (Fig. 4). The isopleths are arbitrary units scaled from 0 to 5. See the text for details on the simulations.



## Vertical egg distributions

Egg concentrations were lowest on the inner shelf, being absent from many tows. On the outer shelf, egg concentrations were also low, ranging up to 0.09–0.1 egg/m$^3$ for eggs in stages I and III and larvae. There was a notable bimodal distribution for cod eggs in stages I–III; stage-I eggs peaked in abundance at 25 and 125 m, stage-II eggs at 25 and 175 m, and stage-III eggs at 15 and 175 m depth (Fig. 9). For cod eggs that occurred <100 m depth, the center of mass on the inner shelf was 52.6 m for stage-I eggs and 16.2 m for larvae; eggs in stages II, III, and IV were too low in abundance to reliably estimate the center of mass, but occurred between 25 and 40 m depth. On the outer shelf the center of mass was calculated to be 35.3, 16.5, 21.4, 21.3, and 11.7 m depth for stages I to V, respectively. The relatively shallow center of mass for stage-II eggs resulted from most eggs occurring <20 m depth, although the peak catch occurred between 20 and 30 m

**Fig. 7.** Simulated cod egg trajectories from fertilization to hatch for eggs in good condition simulated from 300 m for the inner (broken line) and from 280 m for the outer (solid line) Newfoundland Shelf. The horizontal line at 50 m depth represents the bottom of the upper mixed layer depth (MLD).

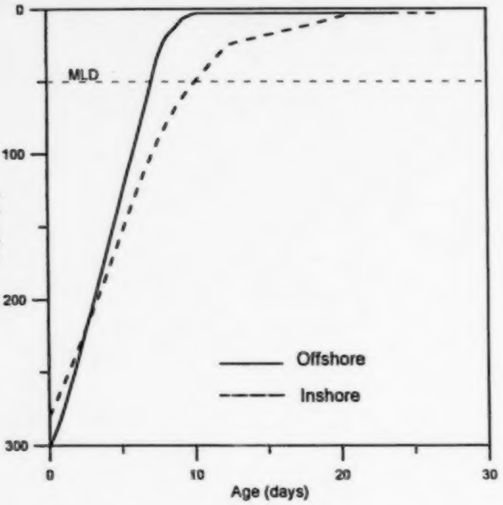
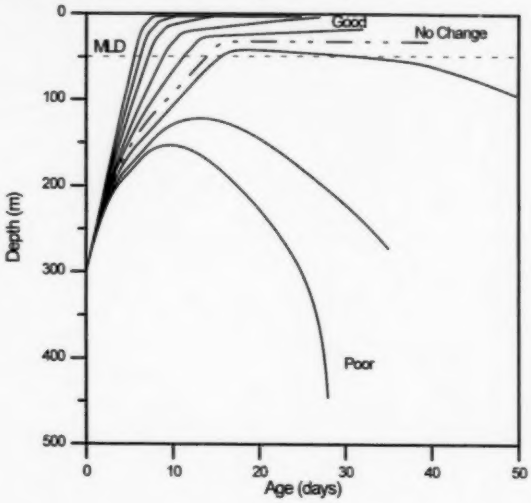


**Fig. 8.** Simulated cod egg trajectories from fertilization to hatch for eggs ranging in condition from good to poor for the outer northeastern Newfoundland Shelf. All eggs started with the same initial buoyancy but the rate of change in egg density was varied from good (decreasing) to poor (increasing). The short–long broken line represents the case where egg density did not change during development. The horizontal line at 50 m depth represents the bottom of the upper mixed layer depth (MLD).



depth. Cod eggs and larvae were deeper in the water column when the inner and outer shelves were compared at a common stage, as expected. However, the relationship to water density was not significantly different (Anderson and deYoung 1994).

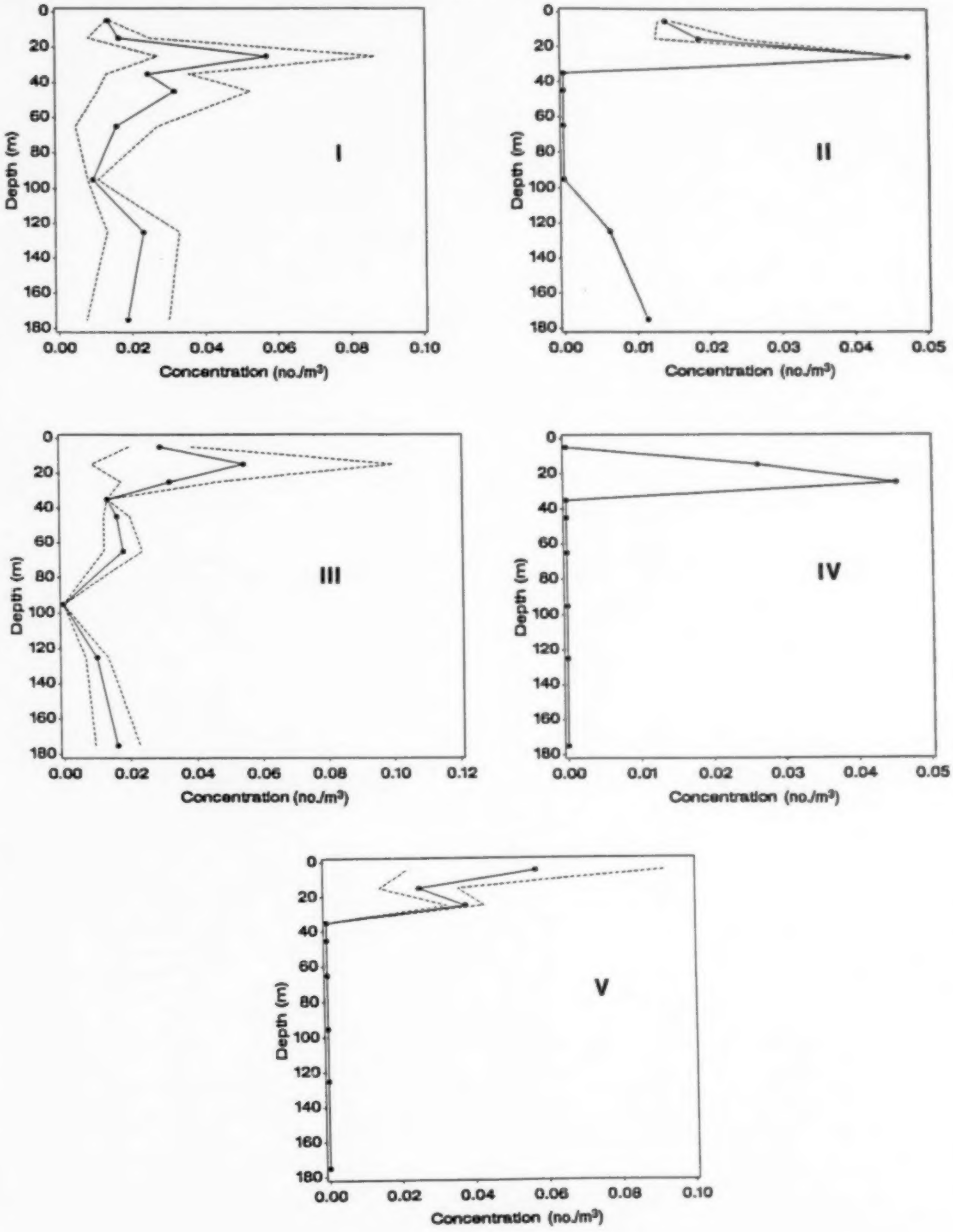
## Discussion

In the laboratory we determined that the velocity formulation of Allen (1900) provided the best fit to the bead and cod egg data. Some previous studies on cod and haddock eggs have used Stokes' formulation (Page et al. 1989; Westgård 1989); others have used Allen's formulation (Sundby 1983). Our results demonstrate that Stokes' formulation significantly overestimates cod egg ascent velocities, compared with Allen's formulation, and that cod eggs will ascend through the water column much more slowly than previously assumed. The model that we have applied does provide a reasonable fit in the very low Reynolds regime expected in the ocean and hence we feel reasonably confident in the modelled results, in spite of the remaining uncertainty in the egg velocity modelling. One should also note that the egg densities that we have determined do not depend on the drag law, since they were determined by observing the point at which the eggs reached neutral buoyancy. However, we believe that the problem of formulating an accurate model of cod egg velocities needs to be explicitly addressed, particularly given the significant effects we found with the different formulations and the fact that cod egg velocities have previously been overestimated.

Water column density structure has a significant effect on the vertical concentrations of cod eggs. We have demonstrated that use of a linear density gradient results in significantly slower egg ascent times and places cod eggs too deep in the water column. Comparison of inner and outer shelf areas also indicated significant differences. On the inner shelf, where waters are less dense, our model predicts that eggs will rise more slowly and will be distributed deeper in the water column for a given developmental stage. This prediction was confirmed by our observations in the field. The sensitivity of vertical concentration profiles to the magnitude and vertical structure of water density indicates that this is an important parameter affecting differences in cod egg ascent times and vertical concentrations.

Previous laboratory measurements demonstrated that cod egg density decreases during development (Anderson and deYoung 1994). This is supported by our field observations where earlier egg stages were found deeper in the water column and larvae were most abundant near the surface. When egg density does not decrease during development our model predicts that cod eggs will not reach the surface and may, in fact, remain deep in the water column. For Northern cod it appears that ascension of cod eggs spawned at 300–600 m depths to surface waters where they will hatch, feed, and survive as larvae may be an important variable affecting survival. Our results contrast with those reported for Norwegian and Baltic cod where initial buoyancies are high and egg density actually increases during development (Sundnes et al. 1965; Mangor-Jensen 1987; Nissling and Westin 1991). For these cod, ascent of eggs to surface waters does not appear to be an important variable affecting survival.

**Fig. 9.** Egg concentrations (no./m³, ±1 SD) for different stages of eggs (stages I–IV) and cod larvae (stage V) measured on the outer Newfoundland Shelf in June 1991.



A complicating factor occurs when poor egg quality affects egg density (Sundnes et al. 1965; Kjesbu et al. 1992; Anderson and deYoung 1994) and reduces buoyancy. In the case of cod on the northeastern Newfoundland Shelf this will result in deeper distributions at a given age. Our model predicts that when egg density increases during development cod eggs will not rise to surface waters and may, in fact, sink. This prediction was supported by field observations, where cod eggs (stages I–III) were abundant deep in the water column.

Relatively high concentrations of cod eggs deep in the water column would be unexpected. Stage-I eggs range from recently fertilized to many days old, and stage I represents approximately 25% of the total egg development time at 3°C (J. Anderson, unpublished data). We estimate that cod eggs ascending on the northeastern Newfoundland Shelf from 300 m depth would take 24–27 d to hatch, indicating that stage-I eggs would be 6–7 d old. Our model predicts that cod eggs in good condition on the outer shelf would be at <100 m depth after 4–5 d and would be concentrated in the surface waters (<50 m) at 7–10 d of age. Therefore, we should expect that only stage-I cod eggs would be found deep in the water column. Stage-II and -III cod eggs sampled at >100 m depth must have been eggs that either were in poor condition and rising slowly through the water column or were, in fact, sinking. Notably, the concentration profiles measured for late-stage eggs (IV) and larvae (V) were unimodal; none was sampled >40 m in the water column. This comparison indicates that by the time cod eggs had reached stage IV they either had ascended to the surface waters where they hatched as larvae or had disappeared from the water column.

The physical characteristics of the water masses in which the Northern cod spawn are known to vary by geographic region, season, and among years. The waters overlying the northeastern Newfoundland Shelf previously have been divided into inner and outer shelf components (Drinkwater and Trites 1986). The inner shelf is characterized by colder, lower salinity water than that on the outer shelf. Temporally, the timing of cod spawning varies from north to south, occurring primarily during April in the northern part of the range when waters are weakly stratified and during June in the southern areas when the water column is seasonally stratified (Myers et al. 1993*b*). There is also significant interannual variability in both temperature and salinity (Petrie et al. 1992). Given the known spatial and temporal variability in water properties and possible interannual variations in spawning distributions (deYoung and Rose 1994) we should expect that cod egg buoyancy will vary among years, even when egg density does not. When egg quality is poorer and egg density greater, then the effects on buoyancy will be more pronounced.

Factors that reduce egg buoyancy will result in slower ascent times to the surface layer. This will reduce transport effects and may have a significant effect on both the dispersal of cod eggs and larvae and where they will settle as juveniles. Failure of eggs to reach surface waters by the time they hatch could have a significant effect on survival. Previous studies have demonstrated statistical relationships between cod recruitment and salinity (Sutcliffe et al. 1983; Myers et al. 1993*a*) and between cod recruitment and temperature (deYoung and Rose 1993). Presently, there is no known direct mechanism to explain these relationships, although some simple models relating survival to spatially variable dispersion of eggs have been suggested (deYoung and Rose 1993; deYoung and Davidson 1994). Changes in water characteristics, including the degree of stratification or the oxygen content of the water, may also adversely affect the vertical distribution and survival of cod eggs.

Previously, cod eggs have been assumed to occur instantaneously in surface waters following spawning. On the basis of this assumption, significant downstream drift has been predicted from transport in surface waters where velocities are highest (Templeman 1981; Lear and Green 1984; Helbig et al. 1992). Our model predicts that eggs in good condition rise from 300 m depth to the surface layer (i.e., <50 m depth) in 7–10 d, would reach the surface in 10–20 d, and would hatch in 24–27 d, on the outer and inner shelf, respectively. These data indicate that ascent time to 50 m depth would comprise 29–37% of total development time, this increasing to 42–78% to reach the surface. The modelled ascent times should have a significant effect on estimates of advective drift and subsequent dispersal. Petrie et al. (1991) characterized the northeastern Newfoundland Shelf as a two-layered system where waters ≤50 m depth are transported downstream at $0.2\ \mathrm{m \cdot s^{-1}}$ and waters below 50 m at $0.1\ \mathrm{m \cdot s^{-1}}$. Assuming a cod egg hatches in 25 d and that ascent time to the surface layer (50 m) varies between 30 and 40% of total development time, then the effect on downstream transport would be to reduce these estimates on the order of 15–20%. While this is a simple calculation, it serves to demonstrate that the effect of cod egg ascent time will significantly reduce the estimates of downstream transport over the northeastern Newfoundland Shelf. When cod eggs are spawned at depths greater than 300 m, as modelled in this study, then the effect on dispersal will be even greater. Future studies modelling the horizontal dispersal of cod eggs on the northeastern Newfoundland Shelf must account for the effect of vertical distributions.

## Acknowledgements

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# Injury to aquatic resources caused by metals in Montana's Clark Fork River basin: historic perspective and overview[1]

Glenn Phillips and Joshua Lipton

## Mining wastes in the Clark Fork River drainage

The community of Butte is located in west-central Montana on the west slope of the continental divide. Copper mining became a viable enterprise in the Butte area about 1882, fueled by the increased demand for copper wire created by the recently invented telephone and electric light (Johnson and Schmidt 1988). Milling, smelting, and ore-concentrating facilities were numerous in and around Butte and many were located along the banks of Silver Bow Creek, the principal tributary to the upper Clark Fork River. These facilities produced a variety of metals-laden waste materials including waste rock, mine tailings, smelter slag, flue dust, and process waters.

Many of these wastes were discharged directly or found their way into Silver Bow Creek and subsequently were carried downstream and distributed throughout the drainageway. Consequently the entire Silver Bow Creek floodplain is contaminated with a fluvially deposited mixture of sediments, tailings, and other mine wastes. Additionally, the upper 200 km of the Clark Fork River are affected by mining wastes present in the stream bed, banks, and floodplain. Andrews (1987) estimated that 99.8 billion kg of mining and smelting wastes were discharged into Silver Bow Creek prior to 1959. Moore and Luoma (1990) estimated that 2–3 million m$^3$ of contaminated sediments are present in the floodplain. Consequently, copper, zinc, cadmium, lead, arsenic, and iron are all at elevated concentrations in stream-bed sediments of Silver Bow Creek and the Clark Fork River (Brook and Moore 1988; Essig and Moore 1995).

Over the first half of this century the mining industry attempted to reduce the movement of mine wastes into the Clark Fork River. A series of three industrial treatment ponds, the Warm Springs ponds, intercept Silver Bow Creek approximately 40 km downstream from Butte. The first and downstream-most treatment pond (pond 1) was built in 1911, the second (pond 2) was constructed in 1916, and the third (pond 3) was completed in 1958 (Johnson and Schmidt 1988). Liming was initiated at the inflow to the ponds in 1959 to aid in the complexation and precipitation of metals originating from upstream sources.

Although the ponds have improved downstream water quality, conditions remain degraded. Water column concentrations of cadmium, copper, lead, and zinc in Silver Bow Creek and the Clark Fork River continue to exceed ambient water quality criteria for the protection of aquatic life (Kerr 1995); copper and zinc concentrations in Silver Bow Creek continuously exceed both acute and chronic criteria. Copper most frequently exceeds criteria in the Clark Fork (Kerr 1995) and is the most toxicologically significant because it is present at the highest concentrations relative to its toxicity (Phillips 1985).

Because of the extensive contamination in the drainage, the U.S. Environmental Protection Agency, in 1983, placed Silver Bow Creek and the upper Clark Fork River on the National Priority List for hazardous waste clean-up sites. Such sites have subsequently become known as Superfund sites and are administered under the Comprehensive Environmental Response, Compensation, and Liability Act. Clean up of the site commenced in the late 1980s and is ongoing. Activities to date have included floodplain tailings removal, in-place liming of tailings, revegetation, improved liming facilities, and increased treatment capacity at the Warm Springs ponds. We anticipate that remediation activities will continue for at least an additional 20 years.

## Fisheries and fish kills

Fish surveys dating back to the late 1800s indicated that toxic mine wastes prevented fish from inhabiting Silver Bow Creek and the upper Clark Fork River. Evermann (1891) reported that "water [in Silver Bow Creek] has the consistency of thick soup, made so by the tailings that it receives from the mills at Butte… throughout the entire length of [the Clark Fork River] the water is full of this solid matter in suspension. The amount of this [material] is considerable, and of course proves fatal to all kinds of fish life. We seined the river very thoroughly in the vicinity of Deer Lodge and did not find any fish whatever."

An electrofishing survey of the Clark Fork near Garrison in 1950 yielded no fish (file memo, Montana Department

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of Fish, Wildlife and Parks, 1420 East Sixth Avenue, Helena, MT 59620-0701). More recently, the Columbia Basin Interagency Steering Committee (1957) concluded that "…from the mouth of Rock Creek to its headwaters the Clark Fork is so heavily polluted by mine wastes that it supports no fishery and any type of development other than pollution abatement would be useless."

With the initiation of liming at the Warm Springs ponds in 1959 a fishery began to establish itself in the upper Clark Fork River. Bob Averett, a biologist with the Montana Department of Fish and Game, found dead fish near the mouth of Rock Creek in 1959 after a "yellow discharge" reportedly emanated from the Warm Springs ponds; throughout the 1960s and early 1970s reports of red or yellow water in the river were frequent (file memos, Montana Department of Fish, Wildlife and Parks). Floods, failure of the Warm Springs ponds dikes, labor strikes that interrupted the liming operation, and heavy winds that resuspended metals precipitates from the bottom of the Warm Springs ponds all contributed to intervals of high concentrations of metals in the Clark Fork River (Johnson and Schmidt 1988). For example, on November 20, 1968, a mining company representative measured 4.0 mg/L copper and 32.5 mg/L zinc in the Clark Fork River below the Warm Springs ponds (file memo, Montana Department of Fish, Wildlife and Parks), concentrations that are greater than 100 times the ambient water quality criteria for protection of aquatic life (U.S. Environmental Protection Agency 1985*a*, 1985*b*).

The U.S. Environmental Protection Agency (1971) concluded that the bottom of the river to Deer Lodge was cemented because of precipitation of gypsum and metallic hydroxides and that there were essentially no fish in the river upstream of Deer Lodge. Metals-contaminated waste discharged from the Warm Springs ponds was implicated as the cause of adverse water quality and bottom conditions.

Between 1972 and 1975 a new waste treatment system at Butte became operational and greatly reduced loadings of metals to Silver Bow Creek and the Warm Springs ponds. The ensuing improvements in water quality of the Clark Fork River resulted in successful invasion and establishment of brown trout (*Salmo trutta*). However, other than for a short distance immediately downstream of the Warm Springs ponds, numbers remain lower than in reference streams having similar habitat (Hillman et al. 1995). Additionally, Silver Bow Creek remains devoid of fish. Hillman et al. (1995) concluded that the absence of fish from Silver Bow Creek and reduced fish numbers in the Clark Fork River were due to the occurrence of metals originating from mine wastes, particularly copper and zinc in sediments, surface waters, and macroinvertebrates.

Fish kills occurred periodically in the Clark Fork River between 1959 and 1975 (file memos, Montana Department of Fish, Wildlife and Parks); however, they were generally of small magnitude because fish populations were low owing to poor water quality. Moreover, the water was so off color that dead fish were difficult to observe. After 1975, the improved waste treatment facilities at Butte and the attendant improvements in water quality of the Clark Fork River downstream of the ponds allowed river trout populations to expand. However, fish kills continued to occur frequently throughout the 1980s and early 1990s, some involving several thousand trout and affecting many kilometres of river. Recent fish kills were caused by thunderstorms that washed metals salts, which had accumulated on the surface of streamside tailings deposits, into the river.

Kills that have been monitored are typified by a sudden drop in pH and alkalinity and a concurrent increase in metals concentrations. Kills were documented in 1983, 1984, 1985, 1988, 1989, 1990, and 1991 (file memos, Montana Department of Fish, Wildlife and Parks).

## Instream toxicity tests

Several investigators have evaluated the toxicity of river water in the Clark Fork drainage. In 1960, biologist Bob Averett held rainbow trout (*Onchorhynchus mykiss*) in cages upstream and downstream of Milltown Reservoir; all fish were dead in less than 3 days and the gills were coated with an orange precipitate (file memos, Montana Department of Fish, Wildlife and Parks). Bionomics (1979) tested the toxicity of water discharged from Warm Springs pond 2 to eggs and fry of rainbow trout: survival of adults and hatchability were not reduced by exposure to pond water but all fry, including those exposed to dilutions of 50 and 75% pond water, showed reduced growth. Copper and zinc concentrations in a 50% dilution of pond 2 water averaged 25 and 65 μg/L, respectively.

Beginning in 1986, instream toxicity tests were conducted in Silver Bow Creek and the Clark Fork River for 4 consecutive years (Phillips and Spoon 1990). Tests with fingerling rainbow trout (1986 and 1987) and swim-up fry (1987–1989) confirmed that toxic conditions continue to occur periodically throughout much of the upper Clark Fork River and almost continuously in Silver Bow Creek; in general, significant mortality occurred during some years at all sites in the Clark Fork upstream of the confluence with Rock Creek as compared with control sites located on Clark Fork River tributaries where metals concentrations are low.

## Overview of current studies

The collection of manuscripts presented in this issue describes part of the work conducted between 1990 and 1993 to evaluate and quantify injury to brown and rainbow trout in Silver Bow Creek and the upper Clark Fork River. This assessment includes food chain toxicity of metals present in macroinvertebrates, waterborne toxicity of simulated pulses of metals, behavioral avoidance of waterborne metals over a range of concentrations, trouts' ability to acclimate to metals, and an evaluation of the physiological impairment of trout exposed to metals in the laboratory or to Clark Fork River water.

Metals in bed sediments and in the water column from the Clark Fork River are accumulated by benthic macroinvertebrates. When these invertebrates were fed to early life stages of trout in the laboratory, growth was reduced and physiological abnormalities were observed (Woodward et al. 1995*a*). Trout fed the contaminated diet experienced gut impaction, cell membrane damage, decreased production of a digestive enzyme, and sloughing of intestinal epithelial cells. Disease and nutritional deficiencies were ruled out as causative agents by pasteurizing and vitamin-fortifying the Clark Fork invertebrate diet. This work confirms that the sediment–macroinvertebrate pathway is an important route of metals exposure for salmonids in the Clark Fork River, and that metals in the diet are toxicologically significant.

Brown and rainbow trout exposed in the laboratory to metals pulses similar to those observed in the Clark Fork experienced significantly reduced survival (Marr et al. 1995*a*). Much of the mortality associated with these pulses was delayed for several days, indicating that fish were irreversibly damaged. Further, under conditions simulating a typical Clark Fork fish kill, rainbow trout were more sensitive than brown trout. Moreover, brown trout juveniles previously acclimated to levels of metals typifying ambient concentrations in the Clark Fork River were more resistant to a metals pulse than similarly acclimated rainbow trout juveniles (Marr et al. 1995*b*). Such differences in relative sensitivity and ability to acclimate to metals may partially explain the presence of brown trout and near absence of rainbow trout in the upper Clark Fork River.

Brown trout actively avoided a mixture of metals similar to that found in the Clark Fork River (Woodward et al. 1995*b*); they also avoided mixtures of 0.5 to 10 times the ambient water quality criteria for the metals mixture. Further, brown trout exposed to progressively higher concentrations of the metals mixture showed a lessened avoidance response, perhaps indicating injury to sensory tissues. Hillman et al. (1995) showed that juvenile and adult trout numbers in reference sites (containing low metals concentrations but otherwise similar to Clark Fork sites) averaged 11 and 36 times those found in the Clark Fork River. The avoidance response of brown trout further explains their reduced numbers in the upper Clark Fork River.

Brown trout from the upper Clark Fork River and hatchery trout exposed to metals in the laboratory experienced elevated concentrations of metals in internal organs, degeneration of digestive cells, and increased lipid peroxidation in several tissues (an index of cellular damage) (Farag et al. 1995; Woodward et al. 1995*a*). Furthermore, brown trout from the upper reaches of the Clark Fork River exhibited higher metallothionein concentrations in liver (a compensatory response to metals exposure) than trout from a downstream location. Microscopic copper inclusions were more common and the pathology was more severe in trout collected below Warm Springs ponds. Farag et al. (1995) conclude that the metabolic cost of metal detoxification protein induction will, at a minimum, result in less net energy available for growth and that all of these stress factors taken together contribute to poorer survival of Clark Fork River trout.

Together, these papers partly describe the mechanisms causing reduced trout numbers and the near absence of rainbow trout in the upper Clark Fork River drainage. Food chain toxicity, waterborne pulses of metals, behavioral avoidance, species-specific acclimation differences, and physiological impairment all influence the numbers, species composition, and distribution of fishes present in Silver Bow Creek and the upper Clark Fork River. While the Clark Fork River and Silver Bow Creek have experienced some recovery over the last 20 years, these studies illustrate the lingering effects of metals contamination and the difficulties of restoring streams when the sediments and floodplain have been contaminated with mine wastes.

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# Metals-contaminated benthic invertebrates in the Clark Fork River, Montana: effects on age-0 brown trout and rainbow trout[1]

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**Abstract**: Benthic organisms in the upper Clark Fork River have recently been implicated as a dietary source of metals that may be a chronic problem for young-of-the-year rainbow trout (*Oncorhynchus mykiss*). In this present study, early life stage brown trout (*Salmo trutta*) and rainbow trout were exposed for 88 d to simulated Clark Fork River water and a diet of benthic invertebrates collected from the river. These exposures resulted in reduced growth and elevated levels of metals in the whole body of both species. Concentrations of As, Cd, Cu, and Pb increased in whole brown trout; in rainbow trout, As and Cd increased in whole fish, and As also increased in liver. Brown trout on the metals-contaminated diets exhibited constipation, gut impaction, increased cell membrane damage (lipid peroxidation), decreased digestive enzyme production (zymogen), and a sloughing of intestinal mucosal epithelial cells. Rainbow trout fed the contaminated diets exhibited constipation and reduced feeding activity. We believe that the reduced standing crop of trout in the Clark Fork River results partly from chronic effects of metals contamination in benthic invertebrates that are important as food for young-of-the-year fish.

**Résumé** : On a récemment imputé aux organismes benthiques du cours supérieur de la rivière Clark Fork la contamination alimentaire par des métaux qui peut constituer un problème chronique pour les jeunes de l'année de la truite arc-en-ciel (*Oncorhynchus mykiss*). Nous avons exposé pendant 88 jours des jeunes de truite brune (*Salmo trutta*) et de truite arc-en-ciel aux premiers stades de développement à de l'eau simulée de la rivière Clark Fork et à un régime d'invertébrés benthiques prélevés dans la rivière. Ce traitement a provoqué une réduction de la croissance et des concentrations élevées de métaux dans la masse corporelle chez les deux espèces. Les concentrations d'As, de Cd, de Cu et de Pb ont augmenté chez les truites brunes entières; chez les truites arc-en-ciel, As et Cd ont augmenté dans le poisson entier, et As a aussi augmenté dans le foie. Chez les truites brunes exposées aux rations contaminées par les métaux, on a observé de la constipation, une surcharge intestinale, un accroissement des dommages aux membranes cellulaires (peroxydation des lipides), une baisse de la production d'enzymes digestives (zymogène) et une desquamation de l'épithélium de la muqueuse intestinale. Les truites arc-en-ciel ont présenté de la constipation et une réduction de l'activité d'alimentation. Nous pensons que la baisse de la biomasse de truite de la rivière Clark Fork est due en partie aux effets chroniques de la contamination par les métaux des invertébrés benthiques qui constituent une nourriture importante pour les jeunes poissons de l'année.

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## Introduction

The upper Clark Fork River has been well characterized for its extensive trace metal contamination in both abiotic and biotic components (Luoma et al. 1989; Moore and Luoma 1990; Axtmann and Luoma 1991; Cain et al. 1992). Bioaccumulation of metals in the benthic community has been used to monitor the degree and extent of contamination in the Clark Fork River (Cain et al. 1992). Benthic invertebrates are an excellent monitoring component: although accumulation of metals from food, water, and sediments is independent and additive, they can tolerate low to moderate metal concentrations (Hare 1992; Timmermans et al. 1992). Moreover, benthic invertebrates are important as food sources for fish and waterfowl and occupy an essential niche in trophic energy transfer and nutrient cycling (ASTM 1993).

The trout fishery in the Clark Fork River above Milltown Reservoir is comprised almost totally of brown trout (*Salmo trutta*) (Johnson and Schmidt 1988; Chapman 1993). Although the habitat should support 1250 fish/km, actual fish densities vary between 21 and 125/km (Johnson and Schmidt 1988). Rainbow trout (*Oncorhynchus mykiss*) inhabit the tributaries but are nearly absent from the main stem of the Clark Fork River above Milltown Reservoir. Age-0 brown trout were 2.4 times more abundant in reference streams than in the Clark Fork River, indicating that recruitment may be limiting trout populations (Johnson and Schmidt 1988; Chapman 1993). Moreover, periodic fish kills from pulses of poor water quality exacerbate the problem. Recent studies indicate that diets of metals-contaminated benthic invertebrates may be a chronic health hazard for young rainbow trout (Woodward et al. 1994). The food-chain effect of metals has been described as a relationship in which biomagnification is not observed and bioconcentration factors are small, but the amount of metal transferred by food can be high enough to attain biologically harmful concentrations in fish (Dallinger et al. 1987). Age-0 brown trout and rainbow trout are more susceptible than older fish because their diets consist totally of drifting benthic invertebrates and zooplankton (Carlander 1969; Hubert and Rhodes 1992). Newly hatched brown trout initially feed on zooplankton but soon shift to small dipterans and ephemeropterans.

The significance of food-chain uptake of metals by fish has been observed in other aquatic systems where, although concentrations in water were low, there was substantial metals contamination of sediments, macrophytes, and benthic invertebrates (Patrick and Loutit 1978; Dallinger and Kautzky 1985; Dallinger et al. 1987; Harrison and Klaverkamp 1989). Investigators have stressed the importance of using environmentally contaminated natural foods (Harrison and Curtis 1992) and the specific ecological situation of a given environment (Dallinger et al. 1987) in assessing effects of contaminants on fish populations. The contamination of benthic food organisms by metals in the upper Clark Fork River appears to be a health hazard to age-0 rainbow trout (Woodward et al. 1994), but additional studies are needed to eliminate variables in diet unrelated to metals, to determine effects on other trout species, to assess contaminated benthic invertebrates from other sources, and to measure health effects in addition to survival, growth, behavior, and tissue metals.

The objective of our study was to characterize diets consisting of benthic invertebrates collected from the Clark Fork River and determine if the differences in metal concentrations would affect survival, growth, behavior, and health of age-0 rainbow trout and brown trout when exposed in water similar to the Clark Fork River. Our study was one of several designed to assess the toxicity of metals-contaminated water and food chain to fish populations of the upper Clark Fork Basin, Montana, U.S.A.

## Materials and methods

### Experimental fish

Eyed embryos of rainbow trout and brown trout were obtained in the fall of 1991 from Ennis National Fish Hatchery, Montana, and Saratoga National Fish Hatchery, Wyoming. Eggs were held in Heath incubators until hatching. Temperature was maintained at 10 ± 1°C during holding and testing, total alkalinity was 150 mg/L, and total hardness was 160 mg/L. Eyed embryos, larvae, and juveniles were handled so as to minimize stress.

### Exposure and control water

Exposure water was formulated at the Jackson Field Station to simulate the Clark Fork River during spring conditions (hardness, 100 mg/L; alkalinity, 100 mg/L; pH, 7.2–7.8). Water quality was analyzed daily to ensure that hardness, alkalinity, conductivity, and pH were within 5% of the desired values. Test water contained the ambient water criteria (1×) concentrations for Cd, Cu, and Pb and was about 50% below the criterion for Zn (U.S. Environmental Protection Agency 1987); that is, 1× = 1.1 μg/L cadmium (Cd), 12 μg/L copper (Cu), 3.2 μg/L lead (Pb), and 50 μg/L zinc (Zn). These concentrations were within the ranges measured in the Clark Fork River (Lambing 1991) and were chosen to simulate conditions in the river and as a regulatory point of reference. No metals were added to the control water (0×).

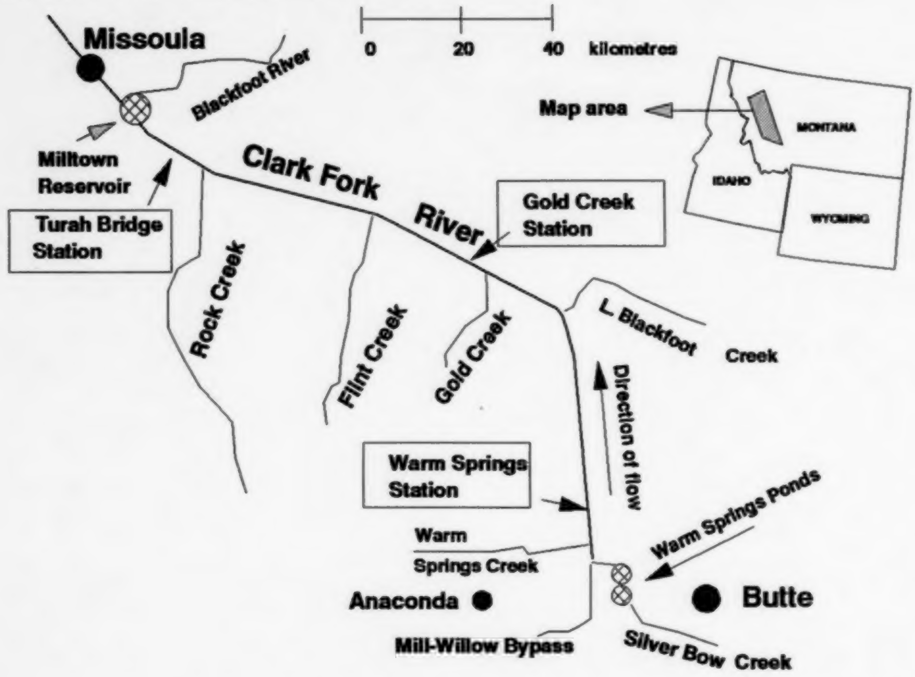
Except for Zn, measurements of all metals in 0× water were below the detection limits of the method, which were as follows (μg/L): Cd, 0.4; Cu, 1.2; and Pb, 1.7. The means of the measured concentrations in the 1× water were within 20% of the nominal value for Cu, Pb, and Zn. Measurements for Cd were 70–85% of the nominal value.

### Exposure diets

Benthic macroinvertebrates were collected from the upper Clark Fork River, Montana, in the spring of 1991. The collection stations (Fig. 1) were 2 km below Warm Springs Creek (WS), 5 km below Gold Creek (GC), and 2 km above Turah Bridge (TB). These stations were selected to represent a gradient in metals concentrations in benthic macroinvertebrates with downstream distance from the source at Butte and Anaconda. The stations at WS and TB also correspond to those used in a previous study (Woodward et al. 1994). Suspected contaminants associated with food-chain organisms from the stations were As, Cd, Cu, Pb, and Zn. The TB station, located about 200 km downstream from the source, provided invertebrates used as the uncontaminated reference diet.

Invertebrates from the three locations were frozen immediately on dry ice and transferred to the Fish Technology Center, Bozeman, Montana, where they were prepared into dry

**Fig. 1.** Upper Clark Fork River from confluence of Silver Bow Creek, Warm Springs Creek, and Mill–Willow Creek to Missoula, Montana, U.S.A., with collection stations identified. Contaminant sources are at Butte and Anaconda.



diets. Procedures previously described (Woodward et al. 1994) were used to eliminate disease from the food organisms and to assure that sufficient vitamins and minerals were present. Invertebrates collected from WS, GC, and TB were pasteurized at 75°C for 15 min and pelletized with a 3% vitamin and mineral premix supplement and a 2% binder similar to procedures used in standard fish feed formulation (Piper et al. 1982). After processing, the diets were dried with an ambient 21°C forced-air drier, and protein, fat, ash, and moisture contents were determined (Woodward et al. 1994).

### Experimental procedure

For both fish species, 75 newly hatched alevins were exposed until 88 d after hatching to 1× and 0× water. The 0× and 1× water treatments were assigned in an alternating arrangement to 12 tanks. Each tank received 1 L of water, which was split into four 250-mL fractions and directed into four identical chambers. The four chambers in each tank were assigned two each to rainbow trout and brown trout by using a random numbers table. This resulted in 12 chambers for each combination of water and species. The three diet treatments (WS, GC, TB) were randomly assigned to these chambers four times apiece. The final design consisted of four replicates of each experimental variable (two water exposures, two species, and three diets) for a total of 48 experimental chambers. Dietary exposures started at exogenous feeding (26 d, brown trout; 18 d, rainbow trout) and continued to 88 d post-hatch.

Exposures were conducted in a flow-through proportional diluter system designed to deliver 1 L of the designated exposure water to each of the 12 water treatments. Each experimental chamber contained 4.2 L of exposure water and received 10 volume additions/d. A Micromedic automated pipetting system was used to maintain selected exposure concentrations. Feeding rate was 6% body weight/d, 1% higher than recommended (Piper et al. 1982). This feeding rate was calculated for the chamber having the largest fish size, and the appropriate weight of food was fed to each chamber.

### Biological measurements

For each experimental chamber, brown trout were thinned by random selection to 40 fish at 26 d and 25 fish at 52 d; rainbow trout were thinned by random selection to 40 fish at 18 d and 25 fish at 53 d. At each thinning and at 88 d, length (mm) and weight (mg) were measured on the individual fish removed. Experimental chambers were checked daily for mortality and observations of behavior.

Measurement of feeding behavior followed procedures used in the previous study (Woodward et al. 1994). A 5-min video recording of each experimental unit was used to analyze fish behavior and included a 2-min nonfeeding period followed by a 3-min feeding period. At selected intervals during the study, overhead video recordings were made of a group of five to seven fish from each experimental unit. At this time the fish were temporarily isolated in a 13 × 13 × 15 cm area of the test chamber by a glass partition. Feeding behavior was evaluated by counting the number of strikes directed toward food particles during a 2-min segment of the recording.

At the end of the test, eight fish of each species were selected for histopathological examination from each of the six combinations of water and diet exposures. Whole fish were placed in Bouin's fixative for 24 h, then transferred to 70% ethanol. After dehydration and clearing, whole fish were embedded in paraffin and sectioned at 4 µm. Sections were mounted on slides and stained with hematoxylin and eosin or rhodamine. Gill, liver, kidney, gastrointestinal tract, and pancreas were qualitatively analyzed for general pathology. At least five slides of each fish (four sections per slide) were analyzed.

### Lipid peroxidation

At the end of the test, eight fish were sampled from each of the 48 experimental chambers for measurements of lipid peroxidation and tissue metal concentrations. The samples were immediately frozen in liquid nitrogen and stored at −70°C. Later, each frozen sample was ground by mortar and pestle, and cooled with liquid nitrogen. In preparation for a fluorometric assay, about 200 mg of ground tissue was processed for measurement of lipid peroxidation (Dillard and Tappel 1984). A chloroform–methanol extraction of tissue was followed by the fluorometric measurement of products from lipid peroxidation. The ground sample (200 mL) was put in a glass homogenizer with a 2:1 mixture of HPLC-grade chloroform–methanol added in an amount that was 35 times the weight of the sample (e.g., 7 mL for a 200-mg sample). The tissue was homogenized, an equal volume of water was added, and the mixture was homogenized again. The mixture was vortexed for 2 min and centrifuged at 3000 rpm for 1 min. The chloroform layer was removed and measured at an emission wavelength of 425 nm and excitation wavelengths of 340 and 360 nm. Previous measurements demonstrated that these wavelengths provided the greatest sensitivity and reliability; two excitation wavelengths were used to ensure that the measurements were reproducible. An increased relative intensity in the fluorometric measurement correlates with an increase in products from lipid peroxidation.

### Necropsy assessment

Each fish was examined for unusual external characteristics when length and weight were measured at 88 d post-hatch, including changes in shape, color, and texture of the eyes, gills, head, fins, and body. At least two fish from each experimental unit were given a complete necropsy inspection (Goede 1990). These fish were dissected and the gill, spleen, kidney, liver, and bile were evaluated along with the fat content associated with each organ. Livers for an additional 5–10 fish were evaluated for metals residue and abnormalities.

### Chemical analysis

#### *Water*

Filtered water samples were taken weekly from the 12 water treatments for metal determinations. Water was filtered using a Nalgene 300 filter holder with a polycarbonate membrane with 0.4-µm pores. Filtered samples (100 mL each) were transferred to precleaned, 125-mL I-Chem polyethylene bottles and preserved by addition of 1 mL Ultrex-II nitric acid. Ca, Cu, Pb, and Zn contents were measured using a Perken-Elmer graphite furnace atomic absorption spectrophotometer (AAS); Zn content at the 1× treatment was determined by flame AAS.

Twice during the experiment, filtered water samples were collected from one of the 0× WS experimental units at 15, 30, 45, 60, and 120 min after feeding to determine if metals in the diet had an effect on the measured concentration of metals in the water. Measurements for As, Cd, Cu, Pb, and Zn were not elevated above background levels. Therefore, when fish were fed the WS diet, high concentrations of metals in food did not increase the dissolved metal concentration in the water column.

#### *Fish and diet*

Fish were collected for metal analysis at each thinning time and at 88 d. Whole fish were analyzed at each collection and liver was also analyzed at 88 d. Fish were not fed 24 h before sampling. Each diet was sampled four times during the study for metals analysis. Three additional diet samples were collected before the start of the study to determine percent moisture, protein, fat, and ash.

On the basis of the results of past analyses (Woodward et al. 1994), five elements were selected for analysis in the diets: As, Cd, Cu, Pb, and Zn. In fish, we measured As, Cd, Cu, and Pb. The method's detection limits for these metals (in µg/g) were as follows: As, 0.74; Cd, 0.27; Cu, 0.31; Pb, 0.88; and Zn, 4.55.

Fish and diet were analyzed with inductively coupled plasma-mass spectroscopy (ICP-MS) on a Perkin-Elmer Elan 5000. Whole fish and liver tissues were lyophilized, digested in a microwave oven, and brought up to a 50 mL final volume in a 5% $HNO_3$ matrix. Initial calibration verification, continuing calibration verification, low-level (CDRL) standard verification, and spike and duplicate measurements were used to verify the analytical method and followed procedures outlined by the manufacturer.

### Data analysis and statistics

Percent survival, growth, physiological, histopathological, and behavioral data were statistically evaluated using two-way analysis of variance. Percentage data were arcsine and square-root transformed, and behavioral count data were square-root transformed before analysis. Since the amount of variation within like treatments showed minimal chamber variation, the data for each species were separately analyzed as a completely randomized design in which the model compared the effects of water, diet, and water times diet (Mark Ellersieck, University of Missouri, Mathematical Sciences Department, Columbia, Mo., personal communication). Treatment differences were ascertained using Fisher's least significant difference (LSD) test (Cochran and Cox 1957). Statistical significance in all tests was assigned at the $P \leq 0.05$ level.

## Results

### Chemical analysis of diets

The three test diets were similar in protein, fat, moisture, and ash content (Table 1). Protein content was between 40 and 50% and lipid content was greater than 15%; both were at or above the recommended limits required for salmonid starter diets at 10°C (Piper et al. 1982). Food energy available for maintenance and growth is usually

**Table 1.** Values for measured constituents in benthic invertebrate diets collected from the Clark Fork River.

| | Constituent measured (%) | | | | Dry weight of metal (mg/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Diet | Protein | Fat | Moisture | Ash | As | Cd | Cu | Pb | Zn |
| TB | 48±0.18 | 16 | 5.9 | 12 | 6.5±0.17 | nd | 87±2.2 | 6.9±0.29 | 616±0.52 |
| GC | 43±0.02 | 15 | 6.4 | 16 | 19±0.15 | nd | 178±5.3 | 15±0.48 | 650±6.1 |
| WS | 44±0.13 | 19 | 8.4 | 12 | 19±0.30 | 0.26±0.52 | 174±1.9 | 15±0.85 | 648±9.1 |

**Note**: Values are given as the mean ± SD. TB, Turah Bridge; GC, Gold Creek; WS, Warm Springs; nd, not detectable. Sample sizes were as follows: % protein, 3; % fat, % moisture, % ash, 2; all others, 4.

**Table 2.** Weight of brown trout through 88 d post-hatch.

| Water diet | Weight (mg) | | |
|---|---|---|---|
| | Day 26 | Day 52 | Day 88 |
| 0× | | | |
| Turah Bridge | 74±3.3*a* | 175±12*a* | 568±22*a* |
| Gold Creek | 70±2.5*b* | 107±5.8*b* | 347±9.7*b* |
| Warm Springs | 68±1.5*bc* | 112±3.2*b* | 344±25*b* |
| 1× | | | |
| Turah Bridge | 68±1.9*bc* | 130±10*c* | 421±59*c* |
| Gold Creek | 67±1.9*bc* | 94±3.8*d* | 267±27*d* |
| Warm Springs | 66±1.0*c* | 87±15*d* | 285±42*d* |

**Note**: Invertebrate diets were collected from three sites on the Clark Fork River, Montana; 0× water simulates conditions in Clark Fork without metals, and 1× simulates Clark Fork with metals. Within the same day, means with the same letter are not significantly different (LSD, $P \le 0.05$). Values are given as the mean ± SD.

**Table 3.** Weight of rainbow trout through 88 d post-hatch.

| Water diet | Weight (mg) | | |
|---|---|---|---|
| | Day 18 | Day 53 | Day 88 |
| 0× | | | |
| Turah Bridge | 94±1.3*a* | 455±10*a* | 1408±56*a* |
| Gold Creek | 94±3.2*a* | 227±14*b* | 758±41*b* |
| Warm Springs | 92±1.7*a* | 224±12*b* | 789±41*bc* |
| 1× | | | |
| Turah Bridge | 92±2.8*a* | 435±16*c* | 1374±45*a* |
| Gold Creek | 92±1.8*a* | 225±3.8*b* | 830±9.1*c* |
| Warm Springs | 92±1.6*a* | 233±14*b* | 801±36*bc* |

**Note**: Invertebrate diets were collected from three sites on the Clark Fork River, Montana; 0× water simulates conditions in Clark Fork without metals, and 1× simulates Clark Fork with metals. Within the same day, means with the same letter are not significantly different (LSD, $P \le 0.05$). Values are given as the mean ± SD.

expressed as kilocalories (1 kcal = 4.18 kJ). From 1 g each of protein, fat, and carbohydrate, there is 3.9, 8.0, and 1.6 cal of energy available (Piper et al. 1982). Using the values in Table 1 for protein and fat, we can calculate the available energy for each diet: 324 kcal in the WS diet, 315 kcal in the TB diet, and 288 kcal in the GC diet. These values represent the energy available for maintenance and growth, and the two test diets (WS and GC) were within 8.5% of the TB reference diet.

When compared with the TB diet, the As content was about three times higher in the GC and WS diets; and the Cu and Pb levels were about two times higher in the GC and WS diets (Table 1). The cadmium content was elevated in the WS diet relative to the GC and TB. The zinc concentration was similar in all diets.

### Survival and growth

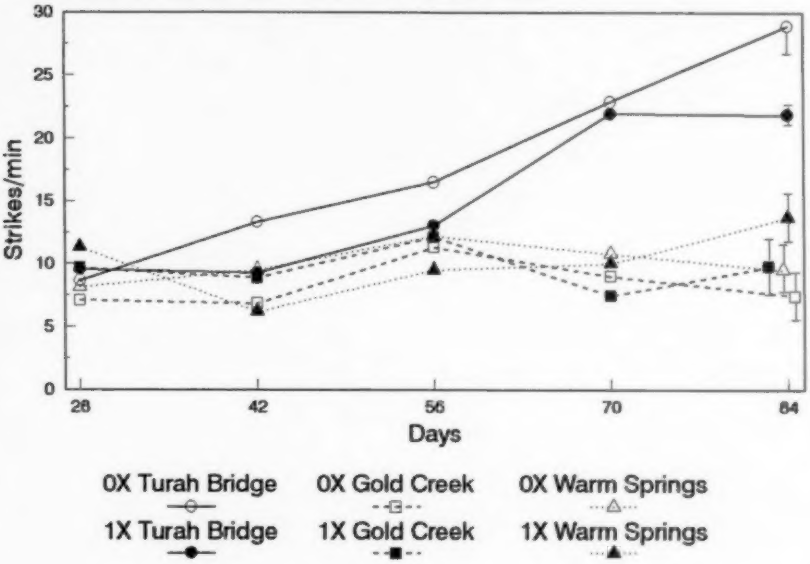
Survival of brown trout and rainbow trout was at or above 88% and was not significantly affected by any combination of water or dietary exposure. Relative to the 0× TB exposure at 88 d, the weight of brown trout was reduced by 25% when the fish were exposed to metals in water alone (1× TB) and was reduced by 40% when exposed to metals in diet alone (0× WS and 0× GC; Table 2). Exposure to metals in both water and diet (1× WS and 1× GC) resulted in a 50% reduction in weight, compared with results of 0× TB exposure. Decreased weight was significant at days 26, 52, and 88. The effects on fish length were similar to those on weight, but were less sensitive and are not reported.

Rainbow trout growth to 88 d was not decreased by exposure to 1× water, but exposure to either the GC or WS diet resulted in a 40–50% reduction in weight at 53 and 88 d when compared with data from fish on the TB diet and in the same water (Table 3). The length of rainbow trout was reduced by about 20% when the fish were exposed to the GC or WS diet, but was unaffected by exposure to 1× water (data not shown).

### Behavior

Brown trout were characteristically slow to begin feeding; thus, the 2-min observation period was not sufficient to monitor the feeding response and detect changes in feeding behavior. Rainbow trout, on the other hand, fed aggressively with little or no latency of response after the introduction of food. Therefore, the 2-min observation period was adequate to document altered feeding behavior in rainbow trout. The feeding frequencies of all rainbow trout averaged nearly 10 strikes/min on day 28 (Fig. 2). Average feeding activity of TB-fed fish steadily increased over the duration of the experiment, from less than 10 to 25 strikes/min, while the feeding activity of fish fed the GC and WS diets

**Fig. 2.** Feeding activity of rainbow trout. Mean number of strikes per minute directed at food during a 2-min observation period, $N = 5$. Rainbow trout fed invertebrate diets were collected from three sites on the Clark Fork River, Montana (Turah Bridge, Gold Creek, Warm Springs); 0× water simulates conditions in the Clark Fork River without metals, 1× simulates conditions in the Clark Fork River with metals.



remained nearly constant over the duration of the experiment. By days 70 and 84 of exposure, feeding behavior appeared to be strongly affected by dietary metal exposure. The feeding activity of rainbow trout fed the GC and WS diets was less than 50% of fish fed the TB diet.

**Bioaccumulation of metals in fish**

*Copper*

For both species, 1× treatment had no effect on Cu concentrations at day 88 (0× TB vs. 1× TB). Within the 0× and 1× water exposures, concentrations of Cu in brown trout after 88 d were significantly higher by 2–3 times for the GC and WS dietary treatments than for the TB treatment (0× TB vs. 0× GC and 0× WS; 1× TB vs. 1× GC and 1× WS) (Fig. 3A). With increased time of exposure (26, 52, and 88 d), Cu concentrations in brown trout continued to increase for all treatments. However, most of the increase was between days 52 and 88.

There were significant increases in Cu concentrations in rainbow trout at 53 d owing to the GC and WS diets. However, at 88 d, there were no significant increases in Cu concentrations in rainbow trout owing to water or diet exposure. The lower concentration at 88 d may be due to the reduction in feeding activity in the WS and GC treatments as compared with TB.

*Arsenic*

Arsenic was not present in the 1× water, and thus did not accumulate in either species receiving aqueous exposure. However, brown trout (at days 52 and 88; Fig. 3B) and rainbow trout (at days 53 and 88; Fig. 4) accumulated significant amounts of As when receiving the GC and WS diets (0× TB vs. 0× GC and 0× WS; 1× TB vs. 1× GC and 1× WS). The magnitude of increase was 3–4 times in brown trout at 88 d and was greater in brown trout than in rainbow trout.

As with Cu, increased time of exposure resulted in increased As concentrations in brown trout and rainbow trout exposed to the GC and WS diets. However, the rate of increase in As concentration in rainbow trout was less between days 53 and 88 than between days 18 and 53, and could probably be accounted for by the reduced feeding activity after 53 d by rainbow trout on the GC and WS diets.

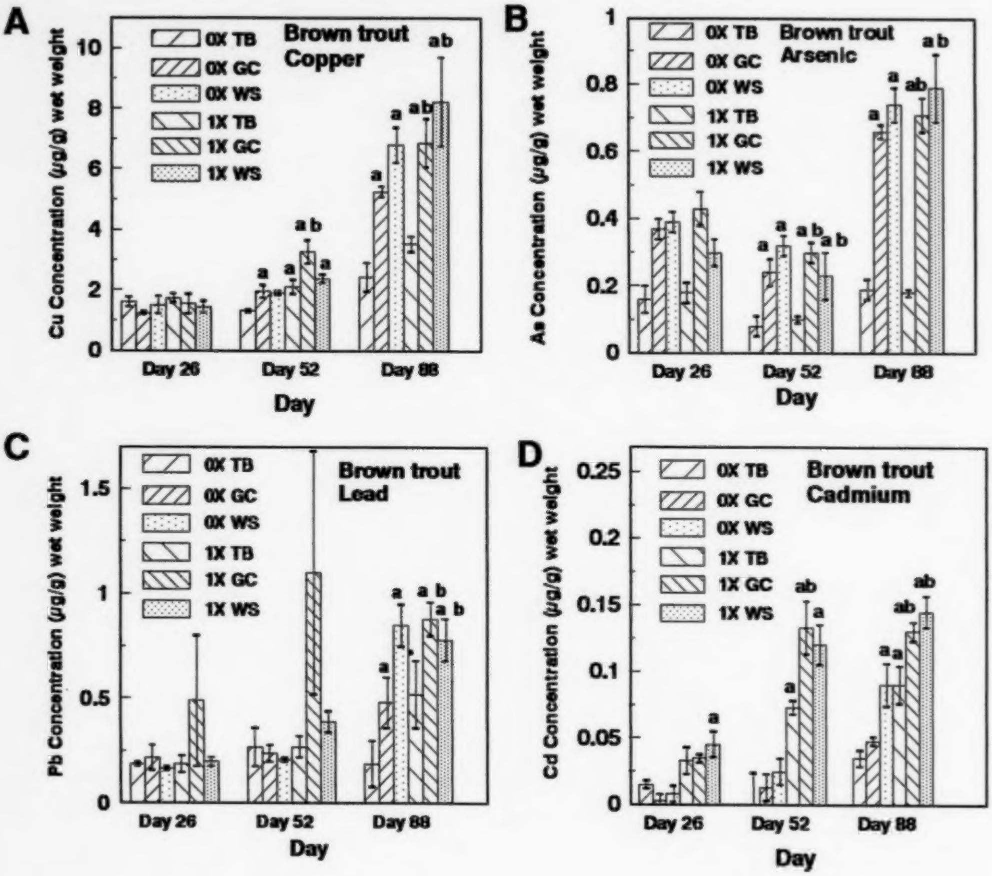
*Lead*

At 88 d, Pb levels in tissue of brown trout were increased after exposure to contaminated water (0× TB vs. 1× TB) (Fig. 3C). Exposing brown trout for 88 d to GC and WS diets resulted in significant increases in Pb concentrations within the 0× and 1× waters (0× TB vs. 0× GC and 0× WS; 1× TB vs. 1× GC and 1× WS). Rainbow trout exposed over the same period exhibited no increases in Pb concentrations.

*Cadmium*

Exposure of brown trout to 1× water resulted in significant increases in the Cd concentration at 88 d (0× TB vs. 1× TB) (Fig. 3D). Exposure to the GC and WS diets resulted in significant additional Cd accumulation over that of 1× water alone at 88 d for brown trout (1× TB vs. 1× GC and 1× WS). Rainbow trout accumulated a significant amount of Cd from the 1× water exposure, but did not accumulate Cd through the diet.

**Fig. 3.** Mean copper, arsenic, lead, and cadmium concentrations (± SE, wet weight) of whole brown trout sampled at three times. Fish were fed invertebrate diets collected from three sites on the Clark Fork River, Montana (TB, Turah Bridge; GC, Gold Creek; WS, Warm Springs); 0× water simulates conditions in the Clark Fork River without metals, and 1× simulates the Clark Fork River with metals. *a*, significantly different from the reference treatment (0× TB); *b*, significantly different from the reference diet and 1× water (1× TB).



*Liver*

Accumulation of metals in liver was more variable than in whole fish. There was a trend of increasing Cu, As, Cd, and Pb concentrations in brown trout livers when the fish were exposed to both water and diet (0× TB vs. 1× GC and 1× WS), but these differences were not significant (data not shown). However, rainbow trout feeding on the GC and WS diets experienced a significant 3-fold increase in liver As content (0× TB vs. 0× WS and 0× GC; 1× TB vs. 1× GC and 1× WS) (Fig. 4).

## Health assessment

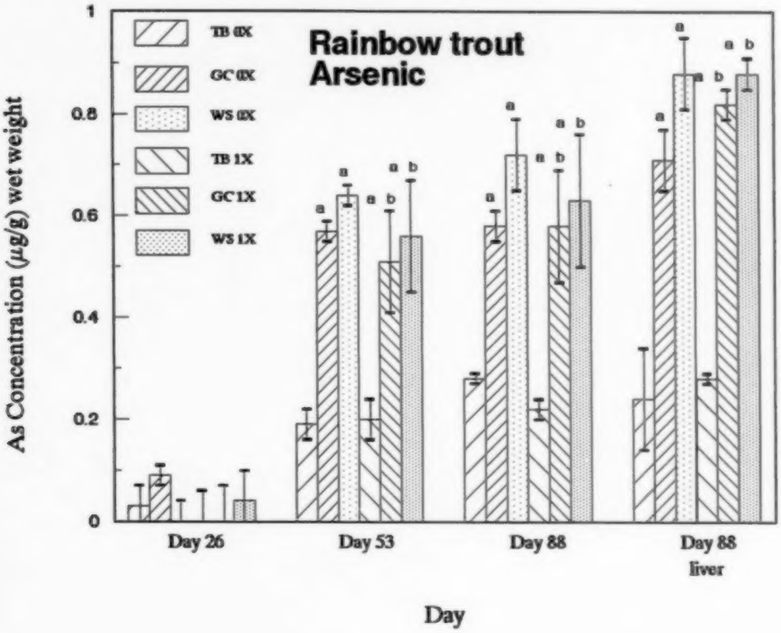
*Histology*

The most significant histological finding for brown trout was noted in exocrine pancreatic tissue of fish fed the diet from WS and GC. When compared with fish fed the 0× TB diet, zymogen granules, precursors of digestive enzymes and commonly found in pancreatic cells, were lacking in seven of eight fish fed the 0× WS diet (Fig. 5) and three of eight fish on the 1× WS diet. The remaining five fish on the 1× WS diet had reduced amounts of zymogen in their pancreatic cells. Swelling and vacuolar degeneration of some pancreatic cells were also noted in fish fed the 0× WS and 1× WS diets.

While degenerative changes consisting of vacuolation and sloughing of intestinal mucosal epithelial cells were seen in brown trout from all treatments they were more severe in fish from the 0× WS and 1× WS treatments (Fig. 6). Few differences were noted in other tissues among treatment groups.

The effects observed on brown trout pancreas and gut epithelium were not observed among rainbow trout. The most obvious histological difference between treatment groups of rainbow trout were in livers between the 0× and 1× waters. Within a diet, livers from fish in the

**Fig. 4.** Mean arsenic concentration (± SE, wet weight) of whole rainbow trout sampled at three times and liver sampled at 88 d. Fish were fed invertebrate diets collected from three sites on the Clark Fork River, Montana (TB, Turah Bridge; GC, Gold Creek; WS, Warm Springs); 0× water simulates conditions in the Clark Fork River without metals, and 1× simulates conditions in the Clark Fork River with metals. *a*, significantly different from the reference treatment (0× TB); *b*, significantly different from the reference diet and 1× water (1× TB).



1× waters exhibited some degeneration of individual hepatocytes and reduced glycogen vacuolation when compared with fish from 0× water.

*Lipid peroxidation*
Brown trout in 0× WS and 1× WS exhibited greater peroxidation than those in 0× TB and 1× TB (Table 4). While the effects of WS dietary exposure were apparent, there was no difference resulting from water exposure (0× TB vs. 1× TB). There was no increased lipid peroxidation in rainbow trout.

*Necropsy assessment*
The most apparent physical deformation observed was the appearance of a swollen abdomen in brown trout. Brown trout exposed to 0× WS and 1× WS diets demonstrated a 4 and 9% occurrence of impaction in the gut, whereas brown trout exposed to 0× TB and 1× TB (reference diets) did not demonstrate any gut impaction. Brown trout exposed to the 0× GC treatment had a 3% occurrence of gut impaction. Gut impaction was not observed in brown trout from the 1× GC treatment or in rainbow trout from any of the treatments. Further evaluation of the impacted gut revealed a swollen stomach and large intestine owing to excess feed material that was not passing through the gut. The gut impaction appeared to be related to a condition that we recorded during our daily monitoring of the experiment. Brown trout and rainbow trout receiving the WS and GC diets were depositing fecal material in long ribbons in comparison with the shorter and narrower diameter fecal material from fish fed the TB diet. More

**Table 4.** Lipid peroxidation of brown trout and rainbow trout fed invertebrate diets collected from three sites on the Clark Fork River, Montana.

| Water and diet | *N* | Brown trout | Rainbow trout |
|---|---|---|---|
| 0× | | | |
| Turah Bridge | 4 | 1.68±0.15*a* | 1.39±0.15*a* |
| Gold Creek | 4 | 2.52±0.06*ab* | 1.22±0.10*a* |
| Warm Springs | 4 | 3.99±0.27*c* | 1.52±0.40*a* |
| 1× | | | |
| Turah Bridge | 4 | 1.80±0.09*a* | 0.94±0.07*a* |
| Gold Creek | 4 | 2.84±0.45*ab* | 1.57±0.23*a* |
| Warm Springs | 4 | 3.85±0.63*bc* | 1.68±0.51*a* |

**Note**: 0× water simulates conditions in the Clark Fork River without metals, and 1× simulates the Clark Fork River with metals. Lipid peroxidation values are given as the mean ± SE and are expressed as the relative intensity of a fluorometric measurement of a chloroform extract of whole fish tissue collected at the end of the study. Within a species, means with the same letter are not significantly different (LSD, $P \leq 0.05$).

**Fig. 5.** Brown trout pancreatic cells after 88 d in water simulating conditions in the Clark Fork River without metals, and either a reference diet (Turah Bridge) or a high metals diet (Warm Springs) of benthic invertebrates collected from the Clark Fork River. (A) Normal pancreatic cells (arrows) showing abundant zymogen granules in cytoplasm of cells (Turah Bridge diet). (B) Pancreatic tissue with numerous vacuoles (arrows) and absence of zymogen granules (Warm Springs diet). ×450.

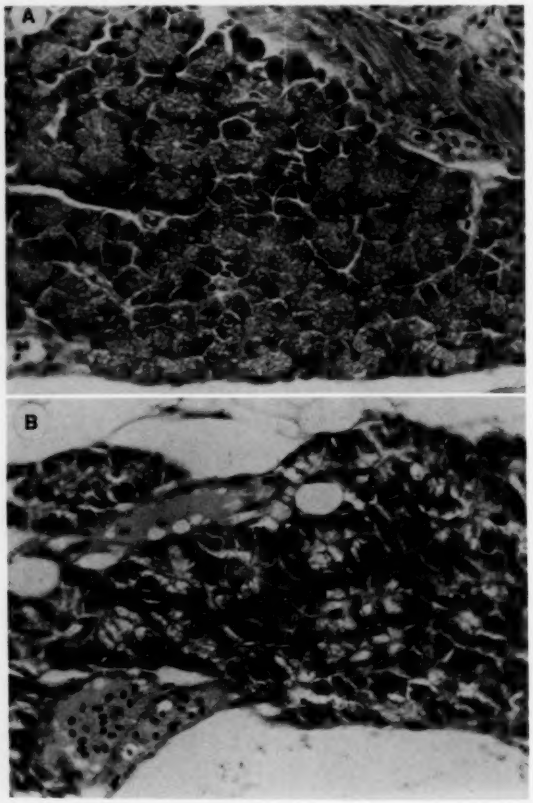
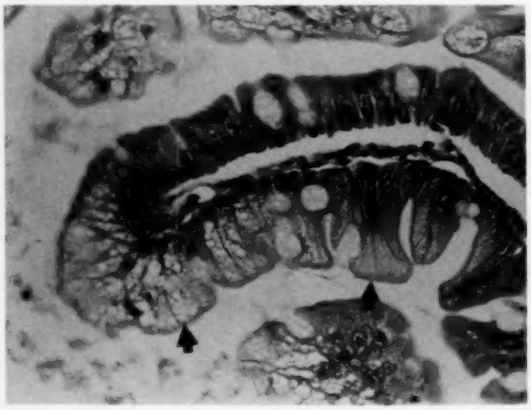


**Fig. 6.** Longitudinal section of brown trout intestine after 88 d in water simulating conditions in the Clark Fork River without metals and feeding on a high metals diet of benthic invertebrates collected near Warm Springs in the Clark Fork River. Note vacuolation and degeneration of intestinal mucosal–epithelial cells (arrows). ×450.



than 50% of fishes receiving the WS and GC diets appeared to be constipated. In the worst cases, as with brown trout, this condition led to impaction in the gut, enlargement of the stomach, and sometimes death. We did not observe any noticeable differences in the color, texture, or size of the other tissues examined.

## Discussion

We have noted widely differing accumulation rates and toxicity reported in studies of metals in fish diets. Experimental dry diets having single ionic metals applied surficially (Wekell et al. 1983; Lanno et al. 1985; Crespo et al. 1986) were less toxic than our diets. One study exposed brine shrimp (*Artemia* sp.) to an aqueous metals mixture for less than 24 h after hatching, and then fed the exposed *Artemia* sp. to rainbow trout (Mount et al. 1994). Metals concentrations in *Artemia* sp. were elevated to concentrations comparable to our field-collected WS diet, but rainbow trout fed on the exposed *Artemia* sp. did not show reduced survival or growth. However, the duration of *Artemia* sp. exposure was so short that the metals were probably in the free form and attached to external surfaces of the *Artemia* sp. It has been demonstrated that ionic metals are not absorbed as efficiently and may not be as toxic as metals bound to proteins (Hodson 1988; Brafield and Koodie 1991; Harrison and Curtis 1992). As, Cd, Cu, and Zn are metabolized in aquatic organisms to organic compounds by covalent bonding or incorporated into proteins by polar or ionic bonding (Craig 1986; Hodson 1988). The present study and our previous study (Woodward et al. 1994) are the only experiments we know of in which both (*i*) multiple metals were associated with the contaminated diet and (*ii*) the diet was made up of food organisms collected from the contaminated system in question.

We did not observe significant reductions in survival as a result of any treatment. However, fishes under toxicological stress in the laboratory may survive because other pressures and hazards of the natural environment are absent. Also, diets were pasteurized and fortified with vitamins and minerals to standardize test diets as much as possible. Pasteurization may change the nature of amino acids and any organo-metal complexes and thus reduce toxicity (Piper et al. 1982). The addition of vitamins and minerals to diets would produce healthier fish that were more resistant to metals than fish on an unfortified diet. For example, vitamins C and E are known antioxidants that could prevent lipid peroxidation of fatty acids (Moslen 1992). In our attempt to rule out causes of mortality other than metals, we altered and probably improved the natural food source.

Results of this experiment agree with previous work where decreased growth and increased levels of metals in tissues were measured in rainbow trout fed a metals-contaminated diet of benthic invertebrates collected from the Clark Fork River (Woodward et al. 1994). In the present study, however, rainbow trout reduced their intake of GC and WS diets after 50 d. That reduction might explain the reduction in both growth and metals concentration. However, As was significantly elevated in both the tissue and the liver of all rainbow trout on the GC and WS diets, indicating that these fish accumulated As from the diet. The occurrence of constipation in rainbow trout from the GC and WS treatments indicated that something more than reduced food intake was affecting health. We believe that metals in natural fish-food organisms may interfere with both food acceptance and fish physiology, resulting in reduced growth and health of the fish.

Constipation and impaction of the gastrointestinal tract have not been previously reported as symptoms in fish exposed to toxic levels of dietary metals. However, symptoms of chronic lead poisoning in mammals include constipation and slowing of nerve conduction in the peripheral nervous system (Dreisbach 1983; Schwartz et al. 1988). The mode of action of lead toxicity in mammals indicates a similar effect on nervous tissue and the gastrointestinal tract of fishes (Scharding and Oehme 1973). Other effects in the gut included a reduction in digestive enzyme precursors (zymogen) and the sloughing of intestinal mucosal epithelial cells in brown trout. The effects on the gut epithelium were similar to the morphological and functional alterations induced in rainbow trout intestine by dietary Cd and Pb (Crespo et al. 1986). The effects on the gut observed in both species in the present study occurred only in treatments where metals contaminated the diets (GC and WS), and probably reduced assimilation and growth.

Heavy metals can be absorbed through the gut and are ultimately distributed to other organs such as liver, kidney, and muscle (Dallinger and Kautzky 1985). We measured an increase in liver As for rainbow trout; reduced growth could result from energy expenditure in binding metals to proteins in the liver, where they are excreted through the bile (Hodson 1988). However, metals may not have to be absorbed to cause reduced growth. The morphological and functional alterations observed in brown trout intestine could decrease assimilation efficiency (Brafield and Koodie 1991).

Our data suggest that contaminants in Clark Fork invertebrate diets are a plausible cause of the decreased survival, growth, and health of age-0 brown trout and rainbow trout. Young-of-the-year fishes in the Clark Fork River depend on a food source of macroinvertebrates, and the metals associated with this food source present a hazard to the sustainability of the fishery. In fact, brown trout collected from the Clark Fork River at Warm Springs had significantly higher tissue concentrations of As, Cd, and Cu and poorer health than fish at Turah Bridge (Farag et al. 1995). Also, trout populations in the Clark Fork River were depressed below those of reference streams and juvenile trout numbers were depressed the most (Chapman 1993). There was a high incidence of liver changes in Warm Springs trout, as demonstrated by copper inclusions stained and observed in hepatocyte cytoplasm; moreover, concentrations of metallothioneins (i.e., metal binding proteins) were elevated in the liver (Farag et al. 1995). Lipid peroxidation products also were greater in tissues of brown trout from Warm Springs. The latter finding is in agreement with the increase in lipid peroxidation levels measured for brown trout fed on the WS diets in our study.

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# Relative sensitivity of brown and rainbow trout to pulsed exposures of an acutely lethal mixture of metals typical of the Clark Fork River, Montana[1]

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**Abstract**: Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) fry and juveniles were episodically or continuously exposed to a metals mixture (Zn, Cu, Pb, Cd): the concentrations and ratios of the metals, and variations in water quality (pH, hardness), were selected to represent conditions measured during episodic storm events in the Clark Fork River, Montana. Brown trout fry were more sensitive (lower $LC_{50}$) than rainbow trout fry to the metals in 8-h exposures with constant hardness and pH, but less sensitive to elevated metal concentrations in conjunction with depressed hardness and pH. Fry were more sensitive than juveniles when exposure was continuous, but neither life stage was clearly more sensitive when exposure was pulsed. Whole-body concentrations of $K^+$ and $Ca^{2+}$ but not $Na^+$ were significantly depressed in fry exposed to metals. Results support the hypotheses that changes in water quality during thunderstorms are lethal to fry and juvenile life stages of brown and rainbow trouts and that the relative sensitivity of the species to the metals mixture may explain their distributions in the Clark Fork River. Low-frequency extreme conditions may effectively act as a bottleneck on the viability of populations whose relative sensitivities to such extremes may control distributions of species in a system.

**Résumé** : Des alevins et des juvéniles de truite brune (*Salmo trutta*) et de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été exposés de façon épisodique ou continue à un mélange de métaux (Zn, Cu, Pb, Cd) : les concentrations et les proportions relatives des métaux, ainsi que les variations de la qualité de l'eau (pH, dureté), ont été sélectionnées de façon à reproduire les conditions mesurées pendant des épisodes orageux dans la rivière Clark Fork, au Montana. Les alevins de truite brune étaient plus sensibles ($CL_{50}$ plus faible) aux métaux que les alevins de truite arc-en-ciel pendant des expositions de 8 h, la dureté et le pH étant constants, mais moins sensibles à des concentrations élevées de métaux coïncidant avec une baisse de la dureté et du pH. Les alevins étaient plus sensibles que les juvéniles quand l'exposition était continue, mais ni aucun de ces stades n'était nettement plus sensible que l'autre quand l'exposition était épisodique. Les concentrations corporelles de $K^+$ et de $Ca^{2+}$, mais non de $Na^+$, étaient nettement abaissées chez les alevins exposés aux métaux. Les résultats confirment l'hypothèse selon laquelle la qualité de l'eau pendant les orages est mortelle pour les stades de l'alevin et du juvénile chez les truites brune et arc-en-ciel, et la sensibilité relative des espèces au mélange de métaux peut expliquer leur répartition dans la rivière Clark Fork. Les conditions extrêmes, de faible fréquence, peuvent jouer efficacement un rôle de goulot d'étranglement sur la viabilité des populations dont les sensibilités relatives à de tels extrêmes peut ainsi régir la répartition des espèces dans un système. [Traduit par la Rédaction]

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## Introduction

Episodic fish kills observed in the upper Clark Fork River, Montana, have been associated with the release of elevated concentrations of toxic metals from tailings deposits and contaminated sediments (Nimick and Moore 1991; Lipton et al. 1995). Zinc, copper, lead, and cadmium are the metals most frequently elevated above water quality criteria in the upper Clark Fork River, a river in west-central Montana affected by mining (U.S. Geological Survey 1989; Moore and Luoma 1990; Lambing 1991). These metals, classified as "very toxic and relatively accessible," are among the metals most toxic to fish (Förstner and Wittmann 1983; Heath 1987). Aquatic biota in the Clark Fork River are subject to three distinct metals exposure regimes: (*i*) chronic exposures to relatively elevated concentrations during spring runoff, (*ii*) chronic exposures to lower concentrations during winter low-flow conditions, and (*iii*) episodic spikes, or pulses of extremely elevated concentrations coupled with concurrent reductions in pH and hardness. These pulses generally have been associated with thunderstorms that mobilize metals from streamside mine wastes (Brooks and Moore 1989). In addition, physical conditions such as ice breakup can resuspend contaminated bed sediments, causing metals pulses in surface water (Johns and Moore 1985). Finally, runoff from streamside acidic bank and floodplain sediments enhances the toxicity of metals by increasing the bioavailable forms, especially for Zn, Cu, and Cd (Ray 1985).

Although concentrations of metals in aquatic systems generally vary temporally, most laboratory toxicity tests are conducted using relatively constant exposures. Water quality criteria developed from continuous exposures may not be adequately protective in natural systems, where episodic or intermittent events can cause changes in both concentrations and speciation of metals (Ingersoll and Winner 1982; Seim et al. 1984; Pascoe and Shazili 1986).

For both fish and invertebrates, pulsed, episodic, or intermittent exposures to toxicants may have a more severe impact than continuous exposure (Ingersoll and Winner 1982; Seim et al. 1984; Pascoe and Shazili 1986; Siddens et al. 1986). For instance, developing steelhead trout intermittently exposed to Cu (4.5 h daily) had lower survival and growth rates and accumulated more Cu than did fish continuously exposed to unchanging equivalent levels of Cu (Seim et al. 1984). A laboratory experiment that measured effects on rainbow trout from exposure to intermittent or continuous Cd concentrations showed that although continuous exposure may reinforce toxicity and accelerate mortality, the initial toxic effects from brief Cd exposures were irreversible and the lethal effect was inevitable (Pascoe and Shazili 1986). In other words, low-frequency extreme conditions may effectively act as a bottleneck on the viability of populations of aquatic organisms. The relative sensitivity of responses to such extremes may therefore control the distribution of species in a system. The objective of this study was to determine the sensitivity of two species of trout to toxic metals when challenged by pulsed and continuous exposures.

## Methods

### Experimental animals

Hatchery brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) were obtained as eyed embryos and as juveniles from hatcheries operated by the Wyoming Game and Fish Department (Dubois Fish Hatchery and Dan Speas Fish Hatchery). Hatchery embryos and juveniles were acclimated to laboratory culture conditions at the Red Buttes Environmental Biology Laboratory, University of Wyoming (see Experimental procedures, below). Following the swim-up stage and throughout pre-exposure acclimation to control water, fry of brown trout (mean ± SE; weight 0.20 ± 0.01 g, length 29.0 ± 0.3 mm) and rainbow trout (weight 0.18 ± 0.01 g, length 28.8 ± 0.2 mm) were fed 5% body weight/d of combined Silver Cup Starter Chow, brine shrimp, and Romet 30 treated chow, and eventually solely Biodiet chow. Juveniles of brown trout (mean ± SE; weight 24.66 ± 0.62 g, length 131.5 ± 1.1 mm) and rainbow trout (mean ± SE; weight 17.80 ± 0.48 g, length 121.5 ± 1.1 mm) were fed 2% body weight/d of a vitamin-fortified commercial trout diet. Fish were acclimated to control water for a minimum of 1 month before testing. Fish condition and health were monitored daily. Thirty-eight hours before each experiment, fish were transferred to exposure chambers and deprived of food. Photoperiod simulated natural light cycles throughout the study period.

### Exposure water and system

Exposure and control waters were formulated by continuously mixing well water (hardness, 220 mg/L as $CaCO_3$; alkalinity, 180–210 mg/L as $CaCO_3$; pH, 7.2–7.8) and deionized water (well water treated with sediment filtration, reverse osmosis, and separate-bed deionization) to simulate spring conditions in the Clark Fork River, Montana (hardness, 100 or 200 mg/L as $CaCO_3$; alkalinity, 80–110 or 180–210 mg/L as $CaCO_3$; pH, 7.2–7.8; temperature, 10°C). Hardness levels exceeding 400 mg/L as $CaCO_3$ (in pulsed exposures) were achieved by metering a $CaSO_4$ stock solution into formulated waters. Fiberglass headtanks (190 L; Frigid Units, Inc.) continuously received the formulated waters, which were adjusted to the desired pH by automatic pH analyzer–controllers (Leeds and Northrup model 7083) using dilute $H_2SO_4$ (these waters were continuously aerated to reduce the concentration of $CO_2$). Temperature and pH were monitored and recorded continuously using a Hewlett-Packard (model 3497A) data-acquisition and alarm system. Exposure and control waters were analyzed daily to ensure that hardness, alkalinity, pH, dissolved oxygen, and temperature were within 10% of the desired levels.

For the pulsed experiments involving only fry, water was delivered to 1-L glass aquaria (filled to 0.5 L) at 25 mL/min to provide an average flow rate of 1500 mL/h to each aquarium and a 90% volume replacement time of <1 h (Sprague 1969). For the pulse experiment involving both juveniles and fry, water was delivered to 340-L circular fiberglass tanks (Figid Unit, Inc.) filled to 56.8 L at 1.9 L/min to provide an average flow rate of 114 L/h to each tank and a 90% volume replacement time of 1.1 h

(Sprague 1969). For the continuous-exposure experiment involving juveniles and fry, water was delivered to 340-L tanks (total volume) at 1.5 L/min to provide an average flow rate of 90 L/h to each tank and a 90% volume replacement time of 8 h (Sprague 1969). For experiments using both life stages combined in the 340-L tanks, fry were placed in suspended enclosures (20.3 × 9.2 × 9.2 cm) made of nitex polypropylene mesh (4 mm diameter mesh) to prevent interactions with juveniles.

Stock solutions of combined metals were prepared from reagent-grade chloride salts ($ZnCl_2$, $CuCl_2$, $PbCl_2$, $CdCl_2$) dissolved and continuously mixed in deionized water. Exposure concentrations of the metals were achieved by metering the stock solution via a Mariotte bottle into continuous-flow diluters. Exposure and control waters were delivered by proportional diluters that provided replicates for each exposure dilution and control.

Exposure waters were identical to the control water, but contained one of four or five geometric dilutions of an 8P or 5P reference mixture during the exposures (P units represent the notation for observed metals concentrations during a documented fish kill in the Clark Fork River), where nominal concentrations for the 1P mixture were 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. The pulsed exposures used four dilutions (8P, 4P, 2P, and 1P) and the continuous exposure used five dilutions (5P, 2.5P, 1.2P, 0.6P, and 0.3P). The metals concentrations (in μg/L) and ratios of Zn, Cu, and Cd in the 1P reference mixture represent dissolved concentrations measured in the Clark Fork River at Deer Lodge, Montana, during a fish kill on July 12, 1989 (Lambing 1991); because measured concentrations of dissolved Pb at the Deer Lodge site were below detection limits on that date, the Pb concentration for the 1P reference mixture was set at 3.2 μg/L, which is the U.S. Environmental Protection Agency's chronic water quality criterion at a water hardness of 100 mg/L $CaCO_3$ (U.S. Environmental Protection Agency 1987).

### Experimental procedures

Fish were acclimated for a minimum of 1 month to the control water used during a given experiment (e.g., hardness, 100 mg/L as $CaCO_3$; alkalinity, 80–110 mg/L as $CaCO_3$; pH, 7.2–8.0; temperature, 10°C). Positions were randomly assigned for the species or life stage to the exposure and control chambers. Individuals of each species and life stage were sequentially transferred in groups of two from holding chambers to exposure or control chambers, thus randomizing the allocation of individuals to the chambers. Ten or 15 individuals were used per exposure dilution and control within each replicate. The initial loading of fish in exposure or control chambers was less than 5.5 g/L.

Mortality, defined as cessation of opercular movement, was monitored every 20–40 min during the pulsed exposure tests and twice daily for 96 h after the pulse; during continuous exposure tests, mortality was monitored every 2 h for the first 12 h and every 6 h subsequently. Exposure and control waters were sampled for metals, temperature, dissolved oxygen, pH, alkalinity, and hardness every 20–40 min before and during pulsed exposures and twice each day after the pulse, or once each day during continuous exposures. Water samples (25 mL) for metals analysis were taken directly from each exposure or control chamber and immediately acidified with 250 μL of 70% $HNO_3$ to preserve the sample until atomic absorption spectroscopy (AAS) analysis was performed. Water samples (50 mL) for pH, alkalinity, and hardness were also taken directly from the exposure or control chamber and analyzed immediately.

For fry receiving pulsed exposure (tests I–V), metals were increased linearly over a 1-h period from control to maximum pulse concentrations, held constant for 6 h, and decreased linearly over a 1-h period to control concentrations (1,6,1-h pulse); the water chemistry was then held constant at control concentrations for the remainder of the experiment for monitoring postpulse mortality. For juveniles and fry receiving pulsed exposure (tests VI and VII), metals were increased linearly over a 2-h period from control to maximum pulse concentrations, held constant for 4 h, and decreased linearly over a 2-h period to control concentrations (2,4,2-h pulse); again, the water chemistry was then held constant at control concentrations for postpulse monitoring. Additionally, during each pulse, the hardness, alkalinity, and pH either remained constant, were depressed, or were increased; changes were linear and occurred over the same time as did changes in metals concentrations. In the text that follows, we use the following abbreviations for exposure conditions according to directions of change and parameters changed: constant hardness and constant pH (CHCp), depressed hardness and constant pH (DHCp), depressed hardness plus depressed pH (DHDp), depressed high hardness plus depressed pH (DHHDp), and elevated hardness and depressed pH (EHDp). Water chemistry was held constant during the continuous exposure. The experimental design for this sequence of tests is shown in Table 1, which includes nominal hardness, alkalinity, and pH during baseline (pre- and post-pulse) and during pulse conditions.

Fish sampled for whole-body $Na^+$, $K^+$, and $Ca^{2+}$ concentrations were frozen in acid-cleaned vials until they were processed for analysis. Individuals were collected at the time of death or at the end of the experiment.

### Analysis of water and tissue chemistry

Concentrations of Zn, Cu, Pb, and Cd dissolved in water were determined by AAS using a graphite furnace or flame (Perkin-Elmer models 2380 and 372). Whole-body ion concentrations ($Na^+$, $K^+$, and $Ca^{2+}$) in fish were determined on a wet-weight basis (Shearer 1984) with AAS using techniques described by Woodward et al. (1989). Blank, spike, standard, and replicate analyses of the same samples used to evaluate quality control were also used to verify instrument calibration and accuracy. Blanks always had element concentrations below instrument detection limits. Spikes introduced at the beginning of sample preparation and spikes added to digestates at the instrument showed an average recovery of >90%, standards generally within 10% of the theoretical values, and values for the second replicate analysis generally within 10% of the first value. Analyses were rejected and samples rerun if values for standards were not within 20% of the theoretical values.

**Table 1.** Nominal and measured water quality conditions from the pulsed and continuous exposures for hardness (ppm $CaCO_3$), alkalinity (ppm $CaCO_3$), and pH (units).

| | | | Baseline conditions | | | | Mid-pulse conditions | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test | Life stage | | Nominal | Average | Range | *n* | Nominal | Average | Range | *n* |
| I (CHCp) | Fry | Hard. | 100 | 104.4 | 100.0–112.0 | 11 | 100 | 108.8 | 104.0–112.0 | 5 |
| | | Alk. | 80–110 | 98.5 | 92.0–104.0 | 8 | 80–110 | 105.0 | 102.0–108.0 | 5 |
| | | pH | 7.2–8.0 | 7.45 | 7.30–7.60 | 8 | 7.2–8.0 | 7.58 | 7.40–7.70 | 5 |
| I (DHCp) | Fry | Hard. | 100 | 105.9 | 96.0–120.0 | 30 | 50 | 64.5 | 44.0–122.0 | 27 |
| | | Alk. | 80–110 | 102.2 | 94.0–120.0 | 24 | 40–60 | 50.3 | 46.0–56.0 | 6 |
| | | pH | 7.2–8.0 | 7.61 | 7.30–7.80 | 30 | 7.2–8.0 | 7.30 | 7.20–7.40 | 6 |
| III (DHDp) | Fry | Hard. | 100 | 102.2 | 93.4–109.6 | 30 | 50 | 47.1 | 40.6–55.0 | 24 |
| | | Alk. | 80–110 | 94.5 | 90.0–102.0 | 15 | 0–10 | 1.4 | 0.9–2.0 | 4 |
| | | pH | 7.2–8.0 | 7.71 | 7.52–7.85 | 30 | 4.5 | 4.84 | 4.45–5.90 | 25 |
| IV (DHHDp) | Fry | Hard. | 200 | 203.4 | 190.8–212.0 | 30 | 100 | 119.4 | 108.0–176.0 | 25 |
| | | Alk. | 180–200 | 186.5 | 182.0–192.0 | 15 | 0–10 | 2.3 | 0.4–8.0 | 14 |
| | | pH | 7.2–8.0 | 7.90 | 7.68–8.09 | 30 | 4.5 | 5.12 | 4.38–6.38 | 25 |
| V (EHDp) | Fry | Hard. | 200 | 207.2 | 196.3–220.3 | 30 | 400 | 423.9 | 403.7–452.9 | 25 |
| | | Alk. | 180–200 | 185.6 | 176.0–192.0 | 15 | 0–10 | 4.2 | 0.9–10.0 | 10 |
| | | pH | 7.2–8.0 | 7.86 | 7.62–8.10 | 30 | 4.5 | 4.96 | 4.61–5..65 | 25 |
| VI (DHHDp) | Fry and juvenile | Hard. | 200 | 204.5 | 195.8–216.2 | 25 | 100 | 110.6 | 106.1–118.3 | 20 |
| | | Alk. | 180–200 | 185.5 | 177.4–192.0 | 13 | 0–10 | 2.2 | 0.97–5.85 | 8 |
| | | pH | 7.2–8.0 | 7.87 | 7.66–8.20 | 25 | 4.5 | 4.83 | 4.25–5.46 | 20 |
| VII (DHHDp) | Fry and juvenile | Hard. | 200 | 207.3 | 199.7–220.5 | 30 | 100 | 134.4 | 108.2–199.7 | 19 |
| | | Alk. | 180–200 | 185.3 | 179.4–191.1 | 18 | 0–10 | 2.9 | 1.95–3.98 | 6 |
| | | pH | 7.2–7.8 | 7.86 | 7.69–8.11 | 30 | 4.5 | 5.05 | 4.48–5.58 | 20 |
| Continuous exposure (CHCp) | Fry and juvenile | Hard. | 100 | 101.5 | 95.9–111.3 | 42 | na | | | |
| | | Alk. | 80–110 | 87.3 | 79.9–91.6 | 14 | na | | | |
| | | pH | 7.2–7.8 | 7.66 | 7.52–7.78 | 42 | na | | | |

**Note**: Conditions of exposure are abbreviated according to directions of change and parameters changed: constant hardness and constant pH (CHCp), depressed hardness and constant pH (DHCp), depressed hardness plus depressed pH (DHDp), depressed high hardness plus depressed pH (DHHDp), and elevated hardness and depressed pH (EHDp). na, not applicable; Hard., hardness; Alk., alkalinity.

Water samples were analyzed for hardness and alkalinity by titrimetric methods (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1985), pH was measured using a calibrated Beckman Omega 12 pH/ISE meter and probes, and dissolved oxygen was measured using a calibrated YSI (model 54A) meter and probe.

### Statistical analyses

To evaluate the sensitivity of fish to the metals mixture, $LC_{50}$ values and 95% confidence intervals were computed using the Spearman–Karber method (Hamilton et al. 1977, 1978). Values for $LC_{50}$ were computed on the basis of maximum average Zn concentrations measured during exposures. Zn data were used for these computations because the Zn concentration was higher than those of Cu, Pb, or Cd in the Clark Fork River and because it could be measured with greater precision in our experiments, again because Zn occurred in higher concentrations. Estimates of $LC_{50}$ were deemed significantly different if 95% confidence intervals did not overlap. This is analogous to a one-tailed test of significant differences at $\alpha = 0.05$ with overall protection of $(0.95)^n$, where $n$ is the number of species or life stages being compared.

In addition, for each species and for each test (experimental design for tests I–V was a completely randomized block; experimental design for tests VI and VII was a completely randomized block with blocks separated by time), relative sensitivity to exposure concentrations was evaluated using a two-way analysis of variance (ANOVA). Specifically, this analysis evaluated effects of metals concentrations and blocks (replicates) on the mean proportion surviving the pulsed exposures and the 96-h postpulse period. The response variable, the proportion of fish surviving, was arcsine square-root transformed (Gelber et al. 1985). Dunnett's multiple comparison procedure (Zar 1984) was conducted to compare survival in each exposure concentration with survival in the corresponding control. Dunnett's comparisons determined the highest concentration of metals (P units) having no statistical difference in survival compared with the control (NOEC, no observed effect concentration) and the lowest concentration of metals (P units) having a statistically reduced survival compared with the control (LOEC, lowest observed effect concentration) (Gelber et al. 1985).

The status of whole-body ions for fish exposed to metals was evaluated using a one-way ANOVA to investigate the effects of survival on the mean concentrations of $Na^+$, $K^+$,

**Table 2.** Measured metals concentrations in exposure dilution (P units) and control (C) waters during the pulsed and continuous exposures.

| Test | Dilution (P units) | Maximum concentration (μg/L) | | | |
|---|---|---|---|---|---|
| | | Zn[a] | Cu[b] | Pb[b] | Cd[b] |
| I | 8 | 2537.6±48.7 | 1245.0±7.1 | 58.12±0.53 | 11.25±0.35 |
| | 4 | 1294.9±68.6 | 601.0[c] | 23.30[c] | 5.80[c] |
| | 2 | 628.1±35.0 | 285.2±4.0 | 11.05±0.14 | 2.63±0.25 |
| | 1 | 321.6±21.8 | 141.0±2.4 | 5.30±0.21 | 1.28±0.04 |
| | C | 22.2±15.8 | nd[c] | nd[c] | nd[c] |
| II | 8 | 2294.5±149.1 | 1323.0±72.1 | 58.88±0.53 | 11.63±0.53 |
| | 4 | 1216.3±39.4 | 680.5±23.3 | 23.85±0.92 | 6.80±0.07 |
| | 2 | 577.0±24.2 | 314.7±14.1 | 10.60±0.78 | 3.48±0.04 |
| | 1 | 283.2±17.8 | 149.2±17.2 | 4.50±0.42 | 1.78±0.00 |
| | C | 23.6±15.0 | nd[c] | nd[c] | nd[c] |
| III | 8 | 2290.8±178.3 | 1478.8±158.4 | 60.63±10.08 | 12.00[c] |
| | 4 | 1151.0±89.8 | 674.8±19.6 | 22.70±0.78 | 6.13±0.04 |
| | 2 | 566.0±20.6 | 404.0±22.6 | 12.68±0.60 | 3.75±0.14 |
| | 1 | 270.7±20.6 | 186.0[c] | 7.00±0.42 | 1.90±0.55 |
| | C | 14.3±13.3 | nd[c] | nd[c] | nd[c] |
| IV | 8 | 2170.3±179.4 | 1286.6±132.1 | 55.50±3.54 | 10.75±0.35 |
| | 4 | 1186.3±120.2 | 708.2±6.4 | 25.05±2.62 | 6.35±0.07 |
| | 2 | 569.4±8.8 | 360.8±16.3 | 14.50±2.19 | 3.77±0.25 |
| | 1 | 300.4±10.5 | 200.7[c] | 7.00±0.28 | 1.68±0.39 |
| | C | 8.6±7.5 | nd[c] | nd[c] | nd[c] |
| V | 8 | 2042.3±251.1 | 1473.2±9.2 | 63.62±2.30 | 12.63±0.18 |
| | 4 | 1040.4±50.2 | 789.0±142.2 | 25.38±3.50 | 6.73±0.18 |
| | 2 | 545.7±8.2 | 399.7±31.5 | 15.00±0.64 | 3.80±0.07 |
| | 1 | 302.6±27.4 | 213.0±8.6 | 8.30±0.00 | 2.18±0.04 |
| | C | nd[c] | 7.2±5.5 | nd[c] | nd[c] |
| VI | 8 | 1814.0±212.7 | 1146.7±23.8 | 31.50±0.71 | 8.80±0.14 |
| | 4 | 947.4±31.8 | 557.6±18.2 | 13.48±0.18 | 3.83±0.04 |
| | 2 | 468.1±42.9 | 291.3±8.1 | 7.25±0.64 | 2.05±0.07 |
| | 1 | 251.1±47.3 | 190.5±39.6 | 3.53±0.04 | 1.10±0.00 |
| | C | 12.7±20.6 | 6.0±0.3 | nd[c] | nd[c] |
| VII | 8 | 1898.8±75.3 | 1146.9±40.1 | 31.75±5.66 | 8.23±0.67 |
| | 4 | 958.3±21.8 | 578.2±16.3 | 14.30±0.07 | 4.08±0.11 |
| | 2 | 466.4±47.7 | 286.6±3.3 | 7.00±0.00 | 1.97±0.18 |
| | 1 | 231.2±5.0 | 145.7±0.1 | 2.98±0.32 | 0.95±0.00 |
| | C | nd[c] | 11.2±0.0 | nd[c] | nd[c] |
| Continuous[d] | 5 | 1187.7±78.4 | 753.0±3.6 | 16.68±0.35 | 13.05±0.35 |
| | 2.5 | 590.8±45.4 | 369.2±11.3 | 8.18±0.11 | 5.60±0.49 |
| | 1.2 | 297.7±19.6 | 180.4±3.9 | 4.05[c] | 3.06±0.06 |
| | 0.6 | 146.5±9.9 | 92.0±3.2 | nd[c] | 1.57±0.04 |
| | 0.3 | 68.6±8.6 | 44.6±0.0 | nd[c] | 0.70±0.00 |
| | C | nd[c] | nd[c] | nd[c] | nd[c] |

**Note**: Values are given as the average ± SD, as determined by atomic absorption spectroscopy.

nd, values below the method detection limit of 4.9 μg/L Zn, 4.6 μg/L Cu, 1.7 μg/L Pb, and 0.4 μg/L cd.

[a] $7 \leq n \leq 16$.

[b] $n = 2$.

[c] $n = 1$.

[d] Average during continuous test.

or $Ca^{2+}$. Tukey's multiple means comparison test was used to detect differences between the mean ionic concentrations in fish that died during exposure and the mean concentrations in both fish that survived exposure and control fish. A type-I error of 0.05 ($p$) was used to judge significance in statistical tests.

## Results

### Water chemistry

Measured hardness, alkalinity, and pH did not deviate from the nominal values by more than 26% in all tests for all characteristics (Table 1). Dissolved oxygen was greater

**Table 3.** $LC_{50}$ estimates and LOEC and NOEC estimates for brown and rainbow trout fry or juvenile life stages from pulsed exposures (8-h pulse + 96-h postpulse observation) and continuous exposure (96 h) to dilutions of a mixture of Zn, Cu, Pb, and Cd.

| | | Brown trout | | | Rainbow trout | | |
|---|---|---|---|---|---|---|---|
| | | $LC_{50}$ (μg Zn/L) | | | $LC_{50}$ (μg Zn/L) | | |
| Test | Life stage | Estimate | 95% CL | LOEC, NOEC (P units) | Estimate | 95% CL | LOEC, NOEC (P units) |
| Pulsed exposures | | | | | | | |
| I (CHCp) | Fry | 1000 | 903–1108*a* | 4P,2P | 1397 | 1222–1597*b* | 4P,2P |
| II (DHCp) | Fry | 454 | 426–484*c* | 2P,1P | 393 | 381–405*d* | 1P,<1P |
| III (DHDp) | Fry | 700 | 639–767*e* | 2P,1P | 386 | 367–407*d* | 1P,<1P |
| IV (DHHDp) | Fry | 1260 | 1142–1390*b* | 4P,2P | 490 | 440–546*c* | 2P,1P |
| V (EHDp) | Fry | >2042 | — | >8P,8P | >2042 | — | 8P,4P |
| VI–VII (DHHDp) | Fry | 1041 | 816–1329*abf* | 4P,2P | 738 | 643–848*ef* | 4P,2P |
| | Juvenile | >1,856 | — | 8P,4P | 691 | 646–739*e* | 4P,2P |
| Continuous exposure | | | | | | | |
| CHCp | Fry | 100 | 93–109*g* | 0.3P,<0.3P | 97 | 87–108*g* | 0.3P,<0.3P |
| | Juvenile | 132 | 118–148*h* | 0.6P,0.3P | 182 | 147–226*h* | 1.2P,0.6P |

**Note**: $LC_{50}$ values are based on the measured Zn concentrations (μg/L) in the metals mixture, where 1P nominal concentrations (μg/L) for the metals were as follows: 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. $LC_{50}$ calculations were made using the Spearman–Karber method (Hamilton et al. 1977). For comparisons, $LC_{50}$ values (μg Zn/L) followed by the same letter are not significantly different, on the basis of comparison of 95% confidence limits (95% CL) ($\alpha = 0.05$) with overall protection of $(0.95)^n$, where $n$ is the number of species or life stages being compared. LOECs and NOECs were determined by Dunnett's comparisons of exposure survival means to control survival means (Gelber et al. 1985). Conditions of exposure are abbreviated according to directions of change and parameters changed: constant hardness and constant pH (CHCp), depressed hardness and constant pH (DHCp), depressed hardness plus depressed pH (DHDp), depressed high hardness plus depressed pH (DHHDp), and elevated hardness and depressed pH (EHDp).

than 75% saturation and temperature remained at 10.0 ± 1.5°C in all exposure chambers for all tests. Maximum average concentrations of Zn were 100–140% of nominal concentrations during the pulsed exposures and average concentrations of Zn were 95–104% of nominal concentrations during the continuous exposure. Concentrations (average maximum, average) and standard deviations measured for Zn, Cu, Pb, and Cd in different tests and dilutions are shown in Table 2.

### Fry pulsed exposure: survival response

Survival was 90–100% for all control fry during the pulse and postpulse periods. Mortality occurred as early as 2 h into the pulse for the highest exposure concentration. Metals exposures as low as 1P and 2P significantly reduced survival in rainbow trout (8-h pulsed exposures) and brown trout (96-h postpulse) when hardness or hardness plus pH were depressed during exposure to metals (Table 3).

Rainbow trout were more tolerant than brown trout under CHCp (i.e., significantly higher $LC_{50}$), but the reverse was true under DHCp, DHDp, or DHHDp (i.e., the $LC_{50}$ was significantly lower for rainbow trout) (Table 3). In all tests with depressed hardness or depressed hardness plus pH, rainbow trout had lower values for $LC_{50}$ and LOEC than when exposed under CHCp (Table 3). Additionally, rainbow trout were less tolerant (lower $LC_{50}$) in tests where hardness was decreased from 100 to 50 ppm as $CaCO_3$ than in tests where the hardness was decreased from 200 to 100 ppm. A similar trend was found for brown trout. Tolerance was clearly greatest (higher $LC_{50}$s and LOECs) when either rainbow or brown trout were exposed under EHDp compared with other pulse conditions.

### Juvenile and fry pulsed exposure: survival response

Survival was 100% for all control fish during the pulse and postpulse periods. Because tests VI and VII used the same exposure conditions and, for each species and life stage, there was no significant difference in survival, these tests were treated as replicates in calculations for $LC_{50}$ and in the Dunnett's comparisons for survival analyses. For rainbow trout at the highest exposure concentration, mortalities occurred as early as 3 h into the pulse for both juveniles and fry, and cumulative survivals were reduced to 0% during the pulse or within 24 h postpulse. For brown trout at the highest exposure concentration, mortalities occurred by 7 h into the pulse for both juveniles and fry, and cumulative survivals were reduced to 60% (juveniles) and 15% (fry) at 96-h postpulse.

$LC_{50}$s for rainbow trout juveniles and fry were similar in the pulsed exposure (Table 3). Although brown trout juveniles had a higher $LC_{50}$ than did fry, because confidence limits could not be calculated for juveniles, statistical significance was not assigned to this difference (Table 3). LOEC values were 4P for brown trout fry and rainbow trout fry and juveniles, and 8P for brown trout juveniles (Table 3). Thus, considering both estimates of $LC_{50}$ and mean survival proportions, the relative sensitivity to pulsed exposures of the two species and two life stages was rainbow trout juveniles ≥ rainbow trout fry > brown trout fry > brown trout juveniles. Therefore, both life stages of the

**Table 4.** Mean whole-body $Na^+$, $K^+$, and $Ca^{2+}$ concentrations (μequiv./g, wet weight) for brown and rainbow trout fry determined from pulsed exposure (test I) using dilutions of the metals mixture of Zn, Cu, Pb, and Cd. Control and exposure survivor groups were sampled at the end of the 8-h pulse + 96-h post-pulse observation and exposure mortality groups were sampled at the time of death.

| | Whole-body ion concentration (μequiv./g) | | | | | |
|---|---|---|---|---|---|---|
| | $Na^+$ | | $K^+$ | | $Ca^{2+}$ | |
| Species and group | Mean ± SE | *n* | Mean ± SE | *n* | Mean ± SE | *n* |
| Brown trout | | | | | | |
| Control | 108.3±10.9 | 38 | 159.9±8.8 | 36 | 570.8±51.3 | 36 |
| Survivor | 103.6±7.5 | 88 | 165.1±7.2 | 87 | 554.8±23.2 | 87 |
| Mortality | 85.5±4.3 | 112 | 115.9±3.5[a,b] | 110 | 385.1±10.4[a,b] | 110 |
| Rainbow trout | | | | | | |
| Control | 53.8±10.2 | 38 | 141.2±15.3 | 38 | 484.9±35.0 | 38 |
| Survivor | 88.1±10.2 | 41 | 127.0±7.5 | 49 | 443.1±35.1 | 49 |
| Mortality | 55.5±3.7 | 42 | 96.1±5.1[a,b] | 41 | 293.0±13.1[a,b] | 41 |

[a] Significantly different ($p < 0.005$) from control.

[b] Significantly different ($p \leq 0.01$) from survivor.

rainbow trout appeared to be more sensitive to pulsed exposures than either life stage of the brown trout.

### Juvenile and fry continuous exposure: survival response

Survival was 100% for all control fish during the continuous exposure. In the highest exposure concentration, mortalities occurred as early as 8 h and survival was at or near 0% at 12 h for all species and life stages. At 96 h, $LC_{50}$ values for both fry species were significantly lower than those for juveniles (Table 3). Whereas the values of $LC_{50}$ and LOEC for brown and rainbow trout fry were nearly identical, the $LC_{50}$ values for juveniles were not significantly different from one another but brown trout juveniles had notably lower $LC_{50}$ and LOEC values than rainbow trout (Table 3).

Finally, $LC_{50}$ and LOEC values from the continuous exposures were lower than for the pulsed exposures for both species and life stages, reflecting the larger time-integrated exposure of metals to fish.

### Whole-body ions

Data for whole-body ion concentrations are restricted to the individual fish from the pulsed exposure of test I. Brown and rainbow trout fry that died during the pulsed exposures had significantly decreased concentrations of whole-body $K^+$ and $Ca^{2+}$, but not $Na^+$ (Table 4). Relative to controls, both brown and rainbow trout fry that died exhibited mean losses of $K^+$ and $Ca^{2+}$ of ≥27 and ≥33%, respectively. When data from fish that survived exposure to metals were compared with data from control fish, no significant differences were found in whole-body concentrations for any of the measured ions.

## Discussion

Episodic fish kills have been observed in the Clark Fork River coincident with spring and summer thunderstorms that release elevated concentrations of Zn, Cu, Pb, and Cd from contaminated floodplain, bank, and, potentially, bed sediments (Nimick and Moore 1991; Lipton et al. 1995). The set of experiments described in this study demonstrates that pulsed exposure to elevated Zn, Cu, Pb, and Cd concentrations, within the range of concentrations and ratios observed during fish kills in the Clark Fork River, adversely and significantly affected survival in both fry and juveniles of brown and rainbow trout. For experiments during which maximum concentrations of metals were present for 6 h or less (8-h exposures), metals concentrations as low as 1P for rainbow trout and 2P for brown trout adversely affected fry survival (see Table 2 for measured metals concentrations). Thus, results from both this laboratory simulation study and from the field support the conclusion that short-term pulsed exposures to metals cause mortality in brown and rainbow trout in the Clark Fork River, Montana.

Additional data emphasize the toxicity of the metals mixture and support the conclusion that episodic events in the Clark Fork produce toxic conditions; significant mortality occurred during or after pulsed exposures in all species and life stages tested. For example, although rainbow trout fry survived the 8-h pulse, none survived the 96-h postpulse observation in the 2P exposure concentration (test II). Pascoe and Shazili (1986) made similar observations; whereas there was no mortality in rainbow trout fry during brief pulsed exposures to Cd (1.0 mg/L for 32 min), mortality after transfer to control water was 50% by 8 d.

As described in Lipton et al. (1995), rainbow trout are uncommon in the more contaminated, upstream reaches of the Clark Fork River. This study and additional studies (Marr et al. 1995; Woodward et al. 1995) examined whether this observed species distribution is consistent with the relative sensitivities of the two trout species to metals. To evaluate the relative sensitivity of the trout species to a metals mixture typical of Clark Fork River pulses, we compared $LC_{50}$ values computed from the 8-h pulsed exposures (+96-h postpulse observation). Under conditions of acutely lethal metals pulses, the relative sensitivity of the two species was altered dramatically if the water quality involved in the exposure was changed. When water hardness and pH were held constant brown trout were more sensitive than rainbow trout, but under conditions more typical of a thunderstorm (i.e., depressed hardness and pH along with elevated metals concentrations) the relative sensitivity to the metals pulse was reversed, with rainbow trout being the most sensitive. As noted in Marr et al. (1995), this response of rainbow trout to complex stress was also observed in acclimation experiments in which brown trout demonstrated apparent enhanced physiological capability, relative to that of rainbow trout, to acclimate to sublethal metals exposures.

The magnitude, duration, and frequency of episodic events have all been found to be important in affecting response to toxicants, especially in more sensitive species. For example, the effect of pH on brook trout was related to the number and magnitude of the acidic episodes (Cleveland et al. 1991), and postpulse mortality in rainbow trout briefly exposed to 1–100 mg Cd/L depended upon the duration of exposure (Pascoe and Shazili 1986). We also found that rainbow trout were more sensitive to exposure duration. This is additional evidence suggesting that, in the Clark Fork, rainbow trout fry are more sensitive than brown trout fry to metals at any duration of pulsed exposure.

We also assessed the relative sensitivity of two life stages (juvenile and fry). Again, the results were dependent on conditions of exposure; for brown trout, juveniles appeared to be only slightly less sensitive (higher $LC_{50}$, no statistical analysis) to pulsed exposures than were fry, and no statistical differences were observed for rainbow trout. Under continuous exposures, juveniles of both brown and rainbow trout were significantly less sensitive (higher $LC_{50}$s) than fry. Previous studies also have reported such differences in susceptibility. For example, the sensitivity of rainbow trout to Cu varied according to fish size, with larger rainbow trout being more resistant (Howarth and Sprague 1978; Chakoumakos et al. 1979). Both chinook salmon and steelhead trout showed differences in sensitivity to Zn and Cd, but not to Cu (Chapman 1978). Alevins were the most resistant among the various life stages examined (newly hatched alevins, swim-up alevins, parr, and smolts).

During pulsed exposures, water quality (i.e., hardness, alkalinity, pH) dramatically influenced survival rates of hatchery brown and rainbow trout fry. Survival was reduced when hardness or hardness plus pH were reduced, and survival increased when hardness increased and pH decreased. Greatest sensitivity occurred for both species when hardness was depressed during the pulse (i.e., significantly lowest $LC_{50}$ values in DHCp- or DHDp-pulsed exposures, tests II and III; Table 3). However, the influence of water hardness and (or) pH on sensitivity was different for brown and rainbow trout (compare values for $LC_{50}$ and LOEC among the various conditions of hardness and pH in the pulsed exposures, Table 3).

The effects of pH reductions on toxicity (at least in the presence of reduced hardness) can be seen by comparing the results of tests II and III (in which the hardness regime was similar). For brown trout, reduced pH during the metals pulse was associated with an increased tolerance to the metals toxicity (higher $LC_{50}$). For rainbow trout, pH reduction had no observable effect on metals sensitivity.

Changes in hardness had a strong inverse effect on metals toxicity (i.e., lower hardness, higher sensitivity). For example, comparing the results of tests I and II demonstrates that brown and rainbow trout were significantly more sensitive to the metals mixture when hardness decreased. Similarly, comparing the results of tests IV and V demonstrates that both species were significantly less sensitive when hardness increased.

The relative effects of initial hardness versus hardness during the pulse are difficult to interpret. Comparing the results of tests I and II versus IV and V (comparisons in which pH was similar) suggests that the hardness during the pulse exposure is most relevant to mitigation of toxicity; in both comparisons, initial hardness was similar and the observed differences in sensitivity were explained by the hardness during the pulse. However, comparison of the results of tests I and IV presents a slightly confounded interpretation. In this comparison, the hardness during the pulse was similar (100 mg/L $CaCO_3$) whereas the initial hardness was different (100 vs. 200); in addition, the pH was lower in the test IV pulse. In this case, brown trout were less sensitive when hardness and pH decreased; this could be explained by the protective effect of reduced pH on brown trout fry. However, rainbow trout were substantially more sensitive when hardness and pH decreased. This increased sensitivity can be explained by changes in initial hardness (again, no effects of pH on rainbow trout sensitivity were observed).

In general, most studies show that metals toxicity is reduced as hardness, alkalinity, and pH increase (e.g., Alabaster and Lloyd 1980; Everall et al. 1989). However, reported effects of pH on metal toxicity are somewhat variable. For instance, in both soft and hard waters (Everall et al. 1989; Bradley and Sprague 1985), Zn was less toxic to juvenile rainbow and brown trout at lower rather than higher values of pH. Zn also was less toxic to steelhead trout fry in soft water (2.3 mg Ca/L) as pH decreased from 7.0 to 5.7 to 4.7 (Cusimano et al. 1986).

Two common mechanisms by which the toxicities of most metals are influenced by chemical properties (i.e., pH, alkalinity, hardness) of the exposure water are (*i*) control of chemical speciation of metals, which determines the relative proportion and availability of different metals, and (*ii*) alteration of metal adsorptivity to cell membranes, which in turn can influence biological responses. Campbell and Stokes (1985) suggested that reduced pH may affect the overall toxicity of metal cations via both mechanisms, i.e., influencing metal speciation by increasing bioavailability of the unbound-cationic species, and also influencing biological sensitivity to metals by decreasing cell membrane adsorption of metals. It should be noted, moreover, that pH – metals speciation relationships (and hence bioavailability) may be complicated by pH changes at the gill surface (gill microenvironment). Playle et al. (1992) found that the pH in the gill microenvironment can differ from that of ambient water by as much as two pH units. At ambient pH in the range of 7–8, the pH at the gill surface has been found to be reduced by more than one pH unit, whereas at ambient pH in the range of 4–5, the gill surface pH was increased slightly by 0.5 of a pH unit (Playle and Wood 1989). Further, over the pH range 5.5–8 of ambient water, net $H^+$ excretion increased linearly at the gill surface in response to increased ambient pH (Lin and Randall 1993). It is possible that, in our study, the observed increase in metals toxicity for brown trout as ambient pH increased may be a function of both $H^+$ competition and decreased pH at the gill surface.

Alkalinity moderates copper toxicity externally; it influences copper speciation by controlling the equilibrium of toxic forms, $Cu^{2+}$, $CuOH^+$, and $CuOH_2^0$, and the less toxic carbonate forms, $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_3^{2-}$. The carbonate or less toxic form generally predominates in high-alkaline conditions, which is why alkalinity is considered to afford protection for fishes (Chakoumakos et al. 1979; Laurén and McDonald 1986). However, in some cases copper toxicity to rainbow trout depends on the total concentration of copper (that is, the concentration of cupric copper, $Cu^{2+}$, and copper carbonate, $CuCO_3^0$) rather than the concentration of either cupric copper or copper carbonate alone (Shaw and Brown 1974).

Calcium, which typically contributes to hardness, may moderate copper toxicity internally by controlling the permeability of branchial epithelium in gills. Thus, the presence of metals when hardness and alkalinity are low should increase electrolyte loss and water uptake (Eddy 1975; Laurén and McDonald 1986; Everall et al. 1989). Eddy (1975) found that, in the absence of calcium, the potentials of brown trout gill epithelia were more negative than in other species (e.g., goldfish), and that calcium additions immediately restored the normal potential. This suggests that in brown trout, calcium greatly reduces ion loss by reducing membrane permeability to $Na^+$. Everall et al. (1989) also found that high $Ca^{2+}$ (i.e., high hardness) altered the membrane permeability of branchial epithelial cells to zinc in brown trout, so that influx was lowered and efflux was enhanced.

Our results showing loss of whole-body ions suggest that the mode by which metals caused toxicity involved ionoregulatory disturbance. For both brown and rainbow trout fry, significant losses of $K^+$ and $Ca^{2+}$ occurred only in individuals that died from the pulsed exposure (Table 4). Acute toxicity of metals to fish often is attributed to cytological damage to gill epithelia (e.g., Mueller et al. 1991). This in turn may disrupt ionoregulation or specific gill functions (e.g., chloride cell function) that are particularly important to freshwater fishes (Eddy 1981, 1982). For example, sticklebacks exposed for 16 h to approximately 1.0 mg Zn/L demonstrated extensive gill damage 5 d after replacement in control water (Matthiessen and Brafield 1973). Laurén and McDonald (1985) showed that loss of $K^+$, $Na^+$, and $Cl^-$ in rainbow trout was strongly dependent on the exposure concentration of Cu and was due to disruptions of branchial ionoregulatory mechanisms within 2 h of the Cu exposure.

Although significant losses of $K^+$ and $Ca^{2+}$ but not $Na^+$ were attributed to the metals toxicity in the present study, some previous work suggests this might not be expected. For instance, Cu may cause loss of both $K^+$ and $Na^+$ by increasing the permeability of (*i*) apical cell membranes, thereby stimulating passive loss of intracellular $K^+$, and (*ii*) branchial tight junctions controlled by bound $Ca^{2+}$, thereby stimulating loss of $Na^+$ (Laurén and McDonald 1985). In the present experiments, the effects of metals on branchial tight junctions may have been repressed by high concentrations of $Ca^{2+}$ in exposure waters. These waters may have supplied sufficient $Ca^{2+}$ to binding sites and thus prevented passive $Na^+$ efflux.

Calcium protects against respiratory impairment from metal toxicity in several fish species. For example, the presence of calcium significantly reduced Zn toxicity in fathead minnows (Judy and Davies 1979). Conversely, lack of $Ca^{2+}$ (low hardness) increased the acute toxicity of Cd to rainbow trout fry (Calamari et al. 1980). Calcium reduced the permeability of branchial epithelia to water in carp but did not reduce Cu uptake, so here apparently calcium provided protection by stabilizing cell membranes rather than reducing toxicant (Cu) uptake (Gregory and Macfarlane 1981). The effect of $Ca^{2+}$ may involve increased stability of gill-epithelial membranes and cell junctions owing to maintenance of cross links between proteins and other cell-wall structures (McWilliams 1983).

In summary, our results support the conclusion that episodic pulses of metals in the Clark Fork River are lethal to both early and juvenile life stages of brown and rainbow trout. This suggests that episodic acute exposures reduce survival in the wild and lower recruitment of subadults into resident trout populations, thus affecting overall population viability.

Additionally, the results of this laboratory study demonstrated that rainbow trout were more sensitive than brown trout to the metals mixture during pulse events when hardness and pH were depressed. This relative sensitivity appeared, in part, to be (*i*) a function of the relatively smaller protective effect of pH reductions on rainbow trout survival relative to brown trout, and (*ii*) a function of the greater adverse effects of hardness reductions on rainbow trout relative to brown trout. In a companion study (Marr et al. 1995), we demonstrated the apparent increased physiological resilience of brown trout to metals toxicity: enhanced capacity for metallothionein induction and sublethal metals acclimation. The results of our pulsed exposure study suggest that similar physiological distinctions between trout species may play a role in relative sensitivity to acute exposures. Given that metal speciation was similar for brown and rainbow trout for the various exposures, the differential responses to hardness, alkalinity, and pH changes support the hypothesis that physiological distinctions between species (e.g., branchial permeability and ion transport) control toxicity. Further, the concept of bioavailability should be viewed as an operational definition that is controlled by both chemical conditions (e.g., speciation) and physiological conditions.

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# Differences in relative sensitivity of naive and metals-acclimated brown and rainbow trout exposed to metals representative of the Clark Fork River, Montana[1]

**J.C.A. Marr, H.L. Bergman, J. Lipton, and C. Hogstrand**

**Abstract**: Naive and metals-acclimated juvenile brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) were exposed to a metals mixture containing zinc, copper, lead, and cadmium, to compare relative sensitivities to metals concentrations typical of the Clark Fork River, Montana. Differences in the survival responses (tolerance versus resistance) measured for naive fish indicated that the rainbow trout are more tolerant (higher 96-h $LC_{50}$) of the metals mixture, yet the naive rainbow and brown trout showed similarities in resistance (mean time to death). Differences for metals-acclimated fish indicated that brown trout are more resistant. Thus, the relative sensitivity of brown and rainbow trout varies with both the survival response measured and pre-exposure effects of metals on the physiological condition of the animal. Brown trout continued to acquire metals resistance for up to 5 weeks during acclimation to chronic concentrations of the metals mixture. Hepatic metallothionein and copper residue levels were positively correlated in both naive and metals-acclimated trout, and an energetic cost of metals acclimation was evidenced by reduced weight in brown trout acclimated to the metals. In contrast, rainbow trout demonstrated the least degree of acclimation and no significant growth inhibition was observed.

**Résumé** : Des juvéniles, certains novices et d'autres accoutumés aux métaux, de truite brune (*Salmo trutta*) et de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été exposés à un mélange de métaux contenant du zinc, du cuivre, du plomb et du cadmium en vue de comparer leurs sensibilités relatives aux concentrations de métaux typiques de la rivière Clark Fork, au Montana. Les différences dans les réactions de survie (tolérance contre résistance) mesurées chez les poissons novices ont indiqué que la truite arc-en-ciel est plus tolérante ($CL_{50}$ à 96 h plus élevée) du mélange de métaux, mais les truites arc-en-ciel et brune novices présentaient des similarités en matière de résistance (temps moyen écoulé jusqu'à la mort). Les différences chez les poissons accoutumés aux métaux indiquaient que la truite brune est plus résistante. La sensibilité relative de la truite brune et de la truite arc-en-ciel varie à la fois avec la réaction de survie mesurée et avec les effets des métaux avant exposition sur l'état physiologique de l'animal. Les truites brunes continuaient à acquérir une résistance aux métaux pendant une durée allant jusqu'à 5 semaines pendant l'accoutumance à des concentrations chroniques du mélange de métaux. Les concentrations de métallothionéine hépatique et de résidu de cuivre étaient positivement corrélées chez les poissons novices et chez les poissons accoutumés aux métaux, et le coût énergétique de l'accoutumance aux métaux a été mis en évidence par la baisse de poids chez les truites brunes accoutumées aux métaux. Par contre, la truite arc-en-ciel présentait le degré le plus faible d'accoutumance, et on n'a observé chez elle aucune inhibition importante de la croissance.
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## Introduction

Mining and mineral processing has contaminated the Clark Fork River, Montana, with elevated concentrations of Zn, Cu, Pb, Cd, and As (Lipton et al. 1995). Both brown and rainbow trout populations in the upper Clark Fork River are reduced in abundance as well as biomass; brown trout currently represent the dominant salmonid (Don Chapman Consultants 1995). This suggests that brown trout (*i*) may be less sensitive to the prevailing concentrations of metals, and (or) (*ii*) may have a greater ability to acclimate to ambient metals than rainbow trout. In a companion study (Marr et al. 1995), we demonstrated that rainbow trout fry are more sensitive than brown trout fry to episodic pulses of elevated metals coupled with depressed hardness and pH.

Acclimation to pollutants has been referred to as an increased tolerance level acquired during exposure to sublethal concentrations that permits resistance to a pollutant (Klerks and Weis 1987). Tolerance or resistance to toxic metals has been broadly defined as "the ability of individuals to cope with the stress associated with exposure to metals concentrations that are inhibitory or lethal to non-tolerant (nonresistant) individuals" (Mulvey and Diamond 1991). The tolerance level of a fish species or strain (stock) to a given metal or metals mixture can be measured as the concentration of metal that can be tolerated indefinitely without mortality in a certain proportion of the population. The resistance level of a fish species or stock can be measured as the time required to induce mortality in a certain proportion of a population exposed to a given concentration of metal. Sprague (1985) differentiates tolerance and resistance by the survival measures or end points associated with concentration and time, respectively. Thus, for tolerance, normally measured as the time-independent median lethal concentration ($LC_{50}$) or incipient lethal level (ILL), the higher the ILL for a given species or stock, the greater the tolerance to the metal exposure. For resistance, normally measured as the median lethal time ($LT_{50}$) or as the mean time to death (mean TTD), the greater the $LT_{50}$ or the mean TTD for a species or stock, the greater the resistance to the metal exposure.

Sensitivity to metals among fish species is controlled by a number of genetic and phenotypic factors, including gill structure and function and metal detoxification capabilities (McDonald and Wood 1993). Exposure to high concentrations of metals over many generations can, in theory, constitute selective pressure on a population of a fish species, resulting in selection of a less sensitive strain (stock). Certain phenotypic responses achieved through physiological acclimation to metals can be induced in fish when pre-exposed to a sublethal concentration of a metal. The genetic basis for physiological responses to metals confounds the distinction between genetically and phenotypically based sensitivity, yet generally the genetic response is considered the inherited tolerance component and the phenotypic response is considered the plastic component (Mulvey and Diamond 1991).

A well-known physiologically based component for metal acclimation and cytoplasmic-metal detoxification involves the specific metal-binding protein metallothionein (MT). Induction of this low molecular weight, cysteine-rich protein is regulated at the transcriptional level by metal ions (Hamer 1986; Kägi and Schäffer 1988; Bremner and Beattie 1990). Laboratory experiments and field studies have confirmed that MT in fish is induced by exposure to metals such as Zn, Cu, Cd, and Hg (reviewed in Hogstrand and Haux 1991). Once induced, MT can bind metals and control distribution within the cytoplasm and alteration of sensitive proteins, enzymes, and organelles (Cherian and Nordberg 1983; Baer and Thomas 1990). Thus, MT's role to bind and regulate potentially toxic metals presents one physiologically based mechanism for metals acclimation that represents a phenotypic response with a genetic basis.

Metabolic costs of acclimation can reduce an organism's scope for growth (Bayne et al. 1979; Naylor et al. 1989; Calow 1991). In fish, metabolic demands associated with metal detoxification (e.g., increased rates of protein synthesis and degradation) have energetic costs (Hogstrand et al. 1995). Such metabolic demands can divert resources from normal growth processes. Further, the physiological changes permitting metals acclimation may, over the long term, reduce the scope for growth in fish.

Reduced growth associated with acclimation to metals such as Zn, Cu, and Cd may be tied to specific structural (e.g., mucous cell proliferation; Wilson et al. 1994*a*), physiological (e.g., respiratory and ionoregulatory status; Collvin 1984; Wilson et al. 1994*b*; Hogstrand et al. 1994), or biochemical (e.g., MT induction; Sprague 1985; Roch and McCarter 1986; Hobson and Birge 1989) changes that represent the actual mechanisms of acclimation. Moreover, the costs of acclimation associated with reduced growth in fish acclimated to metals can be important to consumers and managers of aquatic resources because of the potential for reduced reproductive potential (e.g., increased egg resorption, decreased egg size, reduced spawning success; McFarlane and Franzin 1978), which could lower recruitment of young fish into a population.

In this study, two experiments were performed to examine the relative sensitivity and capacity for physiological acclimation in two stocks of brown trout (*Salmo trutta*) and one stock of rainbow trout (*Oncorhynchus mykiss*) exposed to a metals mixture at a range of concentrations found in the Clark Fork River. The objectives were (*i*) to evaluate relative sensitivity and acclimation levels in the trout species, (*ii*) to study the possibility that MT serves as part of the mechanism for physiological acclimation to metals, and (*iii*) to evaluate reduced growth as an expressed cost of acclimation.

## Materials and methods

The experiments were conducted at the Red Buttes Environmental Biology Laboratory, University of Wyoming, Laramie.

### Experimental animals

Juvenile brown (mean ± SE; weight 25.03 ± 0.52 g, length 132.5 ± 0.9 mm) and rainbow trout (weight 10.61 ± 0.28 g, length 100.6 ± 0.8 mm) were obtained from Dubois and Dan Speas fish hatcheries, operated by the Wyoming Game and Fish Department. Wild juvenile brown trout (mean ± SE; weight 47.42 ± 1.26 g, length 164.0 ± 1.3 mm) were obtained by electroshocking from the upper Clark Fork River, Montana, by staff of the Montana Department of Fish, Wildlife and Parks. All of the fish were held for a minimum of 1 month in control water (hardness, 100 mg/L as $CaCO_3$; alkalinity, 80–110 mg/L as $CaCO_3$; pH, 7.2–7.8; temperature, 10°C) in 800-L Fiberglass tanks (Frigid Units, Inc., Toledo, Ohio) using a flow-through system with a water flow of 5 L/min, providing nine volume additions per day. Water chemistry, temperature, and flow rates were measured daily on samples taken directly from the holding tanks. During holding and acclimation, the fish were fed 2.0% of wet body weight daily with vitamin-fortified commercial trout diet, and the tanks were siphoned daily to remove organic debris. The fish were not fed 24 h before and during the toxicity experiments. The photoperiod simulated natural light cycles throughout the study period. Juvenile fish were used for all experiments, and for one experiment, fish were cold branded (Mighell 1969) 4 weeks prior to the acclimation exposure to identify different treatment groups.

### Exposure water and system

Exposure and control waters were formulated by continuously mixing well and deionized water (well water treated with sediment filtration, reverse osmosis, and separate-bed deionization) to simulate spring conditions in the Clark Fork River, Montana (hardness, 100 mg/L as $CaCO_3$; alkalinity, 80–110 mg/L as $CaCO_3$; pH, 7.2–7.8; temperature, 10°C). Fiberglass headtanks (190 L; Frigid Units, Inc.) continuously received the formulated waters that were adjusted to the desired pH by automatic pH analyzer–controllers (Leeds and Northrup model 7083) using dilute $H_2SO_4$. Temperature and pH were monitored and recorded continuously with a Hewlett-Packard (model 3497A) data-acquisition and alarm system. Exposure and control waters were analyzed daily to ensure that the water quality parameters were within 10% of the desired levels for hardness, alkalinity, pH, dissolved oxygen content, and temperature.

Stock solutions of combined metals were prepared from reagent-grade chloride salts ($ZnCl_2$, $CuCl_2$, $PbCl_2$, and $CdCl_2$) dissolved and continuously mixed in deionized water. Exposure concentrations of the metals were achieved by metering the stock solution via Mariotte bottles into continuous-flow diluters. For the $LC_{50}$ determinations, exposure and control waters were delivered by a proportional diluter designed to provide duplicate controls and duplicates for each of five geometric dilutions of the metals; average water delivery to each tank at 1.5 L/min provided a flow rate of 2160 L/day and a 90% volume replacement time of 8 h (Sprague 1969). For the acclimation experiments, exposure (acclimation and challenge) and control waters were delivered by separate glass head boxes calibrated to deliver water to each tank at 2.5 L/min, which provided a flow rate of 3600 L/day and a 90% volume replacement time of 5 h (Sprague 1969).

From the diluter and head boxes the water was delivered to randomly allocated exposure tanks, and then to waste treatment. Exposure tanks were 340-L circular Fiberglass tanks (Frigid Units, Inc.) with total volumes of 325 L, each receiving moderate aeration. Exposure system components were cleaned with dilute $HNO_3$ and flushed with control water for at least 48 h, and new exposure and control waters were added 24 h prior to each exposure.

Exposure water dilutions for the $LC_{50}$ determinations contained 5.0P, 2.5P, 1.2P, 0.6P, and 0.3P metals concentrations (P units represent the notation for observed metals concentrations during pulse events in the Clark Fork River), with nominal concentrations for the 1P mixture of 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. Exposure water dilutions for the acclimation studies contained a 0.2 dilution of the 1P metals mixture during the acclimation period (where 0.2P is 46 μg/L Zn, 24 μg/L Cu, 0.64 μg/L Pb, and 0.40 μg/L Cd) and the 1P metals concentrations during the challenge period. The 0.2P metals concentrations were chosen to represent base flow conditions measured in the upper Clark Fork River (Lambing 1991). The selected metals concentrations (in μg/L) and ratios of Zn, Cu, and Cd in the 1P reference mixture represent dissolved concentrations measured in the Clark Fork River at Deer Lodge, Montana, during a fish kill on July 12, 1989 (Lambing 1991). Because concentrations of dissolved Pb were below detection limits on that date, the Pb concentration for the 1P reference mixture was set at 3.2 μg/L, which is the U.S. Environmental Protection Agency's chronic water quality criterion at a hardness of 100 mg/L $CaCO_3$ (U.S. Environmental Protection Agency 1987).

### Experimental procedure

#### *$LC_{50}$ determination*

Individuals of each species or stock (hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout) were randomly allocated to treatments. Each exposure treatment was duplicated and each duplicate treatment included 40 individuals of each species or stock. Initial loading of fish in exposure or control tanks was 5.5 g/L.

Mortality, defined as cessation of opercular movement, was monitored every 2 h for the first 12 h and every 6 h for the remainder of the experiment. Dead fish were removed immediately, and the length and weight of every individual were recorded. Exposure and control waters were sampled daily to determine metals concentrations, temperature, dissolved oxygen content, pH, alkalinity, and hardness. Water samples (25 mL) for metal analysis were taken directly from each exposure or control tank and immediately acidified with 250 μL of 70% $HNO_3$ (Instra-Analyzed grade) to preserve the sample until atomic absorption spectroscopy (AAS) analysis was performed. Water samples (50 mL) for pH, alkalinity, and hardness were also taken directly from the exposure or control tank and analyzed immediately.

#### *Acclimation studies*

Cold-branded individuals of each species or stock (hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout) were transferred in groups of 20 from holding tanks to either acclimation (0.2P) or control tanks. At the onset of the acclimation exposure, 140 individuals per exposure or control of hatchery rainbow and brown trout and 75 individuals per exposure or control of Clark Fork brown trout were used. For each challenge exposure, 30 individuals from each of the 0.2P acclimated and control groups were transferred to the challenge tanks containing 1P metals concentrations. This combined both 0.2P acclimated and control groups (with distinctive cold-branding marks) of a given species or stock into a single challenge tank. Initial loading of fish in acclimation or control tanks was 9.0 g/L; this was reduced by about 20% following removal of the fish used in each challenge exposure.

Challenge I followed 3 weeks in the 0.2P acclimation or control water. Challenge II followed 5 weeks of acclimation to the 0.2P acclimation or control water. Challenge III (using only hatchery stocks) followed 6 weeks of acclimation to the 0.2P acclimation and then was followed by 2 weeks of de-acclimation in control water for the metals-acclimated group, and 8 weeks of exposure to only control water for the control group. Challenge III was conducted to determine whether acclimation was lost following 2 weeks in control water. Clark Fork brown trout could not be tested in the challenge III de-acclimation experiment because insufficient numbers of fish were available.

Throughout the acclimation period, mortality was monitored daily; during each challenge, mortality was monitored every 2 h for the first 12 h and every 6 h for the remainder of the experiment. Dead fish were removed immediately, and the length and weight of every individual were recorded.

Exposure and control waters were sampled to determine metals concentrations, temperature, dissolved oxygen content, pH, alkalinity, and hardness every 3 days during the acclimation periods and daily during the challenge exposures. Water from exposure and control tanks was sampled as previously described for the $LC_{50}$ determination.

Fish sacrificed for MT and tissue metal residue analyses were randomly selected from both acclimation and control tanks at 0, 10, and 20 days into the acclimation and at 14 days following de-acclimation.

### Preparation of metallothionein and tissue metal residue samples

Fish were immediately pithed and the liver was removed. The livers were then rinsed in deionized water and blotted dry, placed in sealed cryostatic vials, frozen in liquid nitrogen, and stored at −70°C. At a later date, thawed livers were individually weighed and homogenized in 50 mM Tris-HCl (pH 8.0, 1°C, at a minimum of 1:3 weight:volume ratio). Aliquots of 500 μL were taken from each homogenate, weighed, and acidified with 3 mL of 70% Instra-Analyzed $HNO_3$ for AAS analysis of Zn, Cu, Cd, and Pb. The remainder of each homogenate was centrifuged at 8800 rpm at 4°C for 10 min. Two 500-μL aliquots were taken from each supernatant, frozen in liquid nitrogen, and stored at −70°C for subsequent MT analysis by a competitive double-antibody radioimmunoassay (RIA) for fish MT. The RIA was developed by Hogstrand and Haux (1990) and later modified for rainbow trout MT by Hogstrand et al. (1994). The MT assay used rabbit antiserum raised against MT for perch (*Perca fluviatilis*) as the first antibody, $^{125}$I-labelled rainbow trout MT as a tracer, and goat anti-rabbit IgG as a second antibody. A 10 000 × *g* supernatant prepared from livers of Cd-injected rainbow trout was used as the MT standard. The MT content of the standard was calibrated against a standard curve prepared from rainbow trout MT (Hogstrand et al. 1994). The working range of the RIA was 10–100 ng rainbow trout MT per assay tube, which corresponds to 0.6–6.0 μg/g liver wet weight. No purified brown trout MT was available so rainbow trout MT was also used as the standard for measurement of MT from brown trout. As there may be structural dissimilarities in the antigenic determinants of MT from both species, the reported values of MT from brown trout in this study should be considered as relative.

### Analysis of water and tissue samples

The concentrations of Zn, Cu, Pb, and Cd in water or tissue were determined by AAS using a graphite furnace or flame (Perkin-Elmer models 2380 and 372). Blank, spike, standard, and replicate analyses of the same samples used to evaluate quality control were used to verify instrument calibration and accuracy. Blanks always had metals concentrations below the detection limits of the instrument; method detection limits were 4.9 μg/L Zn, 4.6 μg/L Cu, 1.7 μg/L Pb, and 0.4 μg/L Cd. Spikes introduced at the beginning of sample preparation and spikes added to digestates showed an average recovery of >90%, standards were generally within 10% of the theoretical values, and values for the second replicate analysis generally were within 10% of the first value. Analyses were rejected and samples rerun if values for standards were not within 20% of the theoretical values. Water samples were analyzed for hardness and alkalinity by titrimetric methods described by the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation (1985). pH was measured using calibrated Beckman Omega 12 pH/ISE meter and probes, and dissolved oxygen was measured using a calibrated YSI (model 54A) meter and probe.

### Statistical analyses

To evaluate relative tolerance of fish to the metals mixture, $LC_{50}$ values and 95% confidence intervals were computed using the Spearman–Karber method (Hamilton et al. 1977, 1978). $LC_{50}$ values were computed using average exposure concentrations (Zn, Cu, Pb, and Cd) analyzed by AAS. For comparison, the Zn- and Cu-$LC_{50}$ estimates were determined to be significantly different if the 95% confidence intervals did not overlap. This is analogous to a one-tailed test of significant differences at $\alpha = 0.05$ with overall protection of $(0.95)^3$. The percent contribution of each metal to the toxicity of the mixture was calculated using methods described by Parkhurst et al. (1981) based on the individual metals concentrations at the 96-h $LC_{50}$ dilution for rainbow trout in this study and 96-h $LC_{50}$ concentrations reported for the individual metals for rainbow trout (U.S. Environmental Protection Agency 1980, 1984*a*, 1984*b*, 1984*c*; Mance 1987).

ILL estimates were calculated for Zn and Cu using linear regression analysis of $LC_{50}$s at successive time intervals to find the best-fit line (i.e., highest adjusted $R^2$ coefficient) for the upper and lower curve components, a modification of the method described by Sprague (1969). Each ILL was calculated by solving simultaneous equations for the two regression functions to determine the lethal threshold time value (LTT, the apparent time of lethal threshold; Sprague 1969), which corresponds to the intersection of the upper and lower curve components, and then using the LTT to solve for the metal concentration representing the ILL.

Results from the acclimation experiments to determine relative resistance to metals exposure were analyzed by a

**Fig. 1.** Zinc and Cu median lethal concentrations ($LC_{50}$) and 95% confidence intervals at 12- to 144-h time intervals for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout exposed to dilutions of a mixture of Zn, Cu, Pb, and Cd. $LC_{50}$ values are based on measured Zn or Cu concentrations (μg/L). $LC_{50}$ calculations were made using the Spearman–Karber method (Hamilton et al. 1977). For comparisons within each time period, $LC_{50}$ values shown with 95% confidence limits overlapping are not significantly different, with overall protection of $(0.95)^3 = 0.86$.

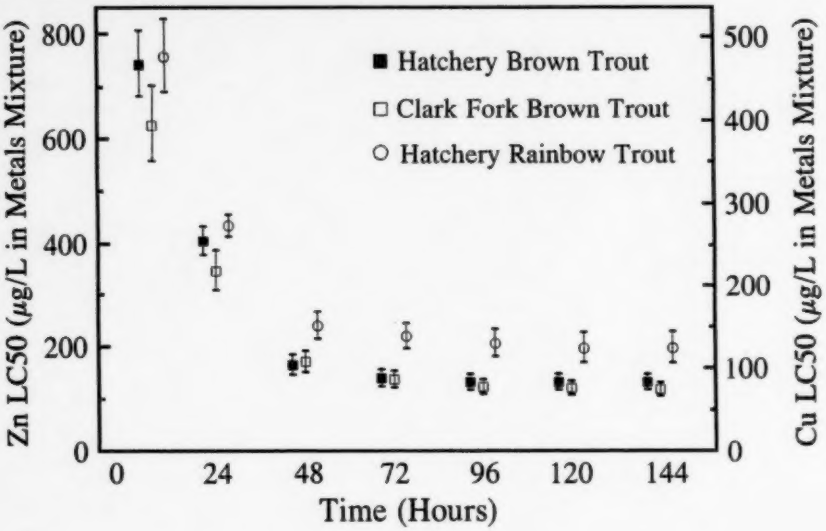


**Table 1.** Water quality conditions measured in the exposure waters during the $LC_{50}$ and acclimation experiments.

| Experiment | Parameter | Nominal | Average | Range | n |
|---|---|---|---|---|---|
| $LC_{50}$ | Hard. | 100 | 101.5 | 95.9–111.3 | 42 |
| | Alk. | 80–110 | 87.3 | 79.9–91.6 | 14 |
| | pH | 7.2–7.8 | 7.66 | 7.52–7.78 | 42 |
| 0.2P Exposure (Acclimation) | Hard. | 100 | 100.4 | 95.7–106.1 | 39 |
| | Alk. | 80–110 | 90.8 | 85.8–95.5 | 14 |
| | pH | 7.2–7.8 | 7.58 | 7.42–7.73 | 39 |
| Control (Acclimation) | Hard. | 100 | 99.8 | 95.7–106.1 | 39 |
| | Alk. | 80–110 | 95.5 | 85.8–95.5 | 12 |
| | pH | 7.2–7.8 | 7.58 | 7.43–7.71 | 39 |
| Challenge I | Hard. | 100 | 100.9 | 97.8–104.0 | 18 |
| | Alk. | 80–110 | 91.0 | 85.8–95.6 | 18 |
| | pH | 7.2–7.8 | 7.56 | 7.48–7.66 | 18 |
| Challenge II | Hard. | 100 | 100.6 | 97.8–104.0 | 25 |
| | Alk. | 80–110 | 91.1 | 83.9–97.5 | 25 |
| | pH | 7.2–7.8 | 7.60 | 7.51–7.72 | 25 |
| Challenge III | Hard. | 100 | 99.2 | 98.0–102.0 | 8 |
| | Alk. | 80–110 | 90.3 | 87.7–93.6 | 8 |
| | pH | 7.2–7.8 | 7.60 | 7.53–7.68 | 8 |

**Note**: Values for hardness (as ppm $CaCO_3$), alkalinity (as ppm $CaCO_3$), and pH are expressed as nominal, measured average, respective range, and sample size (*n*).

two-way analysis of variance (ANOVA), which compared the mean time to death (TTD; hours) for the three species exposed to the two different acclimation levels (0.2P and control). The effects of the size of fish on differences in mean TTD were evaluated by including weight (g) as a covariate in the ANOVA models. The response variable measured on each fish was the number of hours until death, where the value of this response was the midpoint of the interval between the discrete time period where the fish was observed dead and the previous time period observed. For each of the challenge periods, a series of *t* tests (Montgomery 1983) were used to (*i*) compare (a priori) the mean TTD between acclimation levels within each species, and (*ii*) compare (a priori) the mean TTD between species within each acclimation level.

The effect of acclimation to metals on the MT concentration in fish liver was evaluated using two-way ANOVA. The response variable measured on individual fish was the MT concentration in the liver homogenate. Tukey's multiple means comparison test was used to detect differences between the mean MT concentrations of control, acclimated, and de-acclimated groups. Partial correlation analysis (Zar 1984) was performed to identify, in the computed correlation matrix, which metal(s) accumulated (μg/g) in the liver had significant correlation with MT concentration. Finally, Pearson's product-moment correlation (Zar 1984) was used to calculate correlation coefficients between the identified metal and MT concentrations.

To evaluate the effect of acclimation to metals on fish weight for each species and stock exposed to the two different

**Table 2.** Measured metal concentrations in exposure dilution (P units) and control (C) waters during the $LC_{50}$ determinations and during the acclimation and challenge exposures.

| Experiment | P unit | Zn[a] | Cu[b] | Pb[b] | Cd[b] |
|---|---|---|---|---|---|
| LC50 | 5.0 | 1187.7±78.4 | 753.0±3.6 | 16.68±0.35 | 13.05±0.35 |
| | 2.5 | 590.8±45.4 | 369.2±11.3 | 8.18±0.11 | 5.60±0.49 |
| | 1.2 | 297.7±19.6 | 180.4±3.9 | 4.05[c] | 3.06±0.06 |
| | 0.6 | 146.5±9.9 | 92.0±3.9 | nd | 1.57±0.04 |
| | 0.3 | 68.6±8.6 | 44.6±0.0 | nd | 0.70±0.00 |
| | C | nd | nd | nd | nd |
| 0.2P exposure (Acclimation) | 0.2 | 53.0±4.3 | 33.6±4.5 | nd | 0.54±0.09 |
| Control (Acclimation) | C | 2.8±3.8 | 6.4±5.3 | nd | nd |
| Challenge I | 1.0 | 260.8±10.7 | 150.4±7.3 | 3.51±0.70 | 3.03±0.12 |
| Challenge II | 1.0 | 249.1±12.6 | 116.3±9.6 | 3.87±0.32 | 2.88±0.10 |
| Challenge III | 1.0 | 287.7±26.0 | 163.0±3.8 | 4.50±0.71 | 3.45±0.07 |

**Note**: Values are given as the mean ± SD. P units represent the notation for observed metals concentrations during pulse events in the Clark Fork River, where metal concentrations for 1P are 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. Average Zn, Cu, Pb, and Cd (μg/L) concentrations were determined by AAS. nd, values below the method detection limit 4.9 μg/L Zn, 4.6 μg/L Cu, 1.7 μg/L Pb, and 0.4 μg/L Cd.

[a] $8 \le n \le 39$.

[b] $n = 2$.

[c] $n = 1$.

acclimation levels (0.2P and control), two-way ANOVA was used. The response variable measured on individual fish was wet weight. Further analysis involving contrasts with a single degree of freedom (Sokal and Rohlf 1981) was used to determine differences between acclimation and control means at each time period of acclimation or de-acclimation.

Unless otherwise indicated, a type-I error of 0.05 ($p$) was used to judge significance in statistical tests. All statistical analyses were performed using the SYSTAT system for statistics (Wilkinson 1990).

## Results

### Water chemistry

Measured hardness, alkalinity, and pH values were within 6% of nominal concentrations with the exception of water hardness during the $LC_{50}$ determination, which averaged only 1.5% over the nominal value but ranged as much as 11% over the nominal concentration (Table 1). Dissolved oxygen was between 90 and 98% saturation and temperature remained at 10.0 ± 0.5°C in all exposure or control tanks.

The average measured Zn concentrations were 95–104% of the nominal concentrations from the $LC_{50}$ determination and were 108–125% of the nominal concentrations from the acclimation experiments. The average Zn, Cu, Pb, and Cd concentrations and standard deviations for the different exposure dilutions are summarized in Table 2.

### Tolerance to metals: $LC_{50}$ and ILL estimates

Survival was 100% for all control fish during the $LC_{50}$ and ILL determinations. In the highest exposure concentration (5.0P), cumulative mortalities were equivalent for all three species or stocks; mortalities were observed as early as 8 h and survival was at or near 0% at 12 h for all three species or stocks. The $LC_{50}$ values for hatchery and Clark Fork brown trout were similar at each time interval (Fig. 1 and Table 3). Clark Fork brown trout had lower $LC_{50}$s than hatchery rainbow trout at time intervals ≥ 24 h (Fig. 1). Hatchery brown trout had lower $LC_{50}$s than hatchery rainbow trout at time intervals ≥ 48 h (Fig. 1).

The LTTs of the ILLs were between 31 and 33 h for all three species or stocks; no differences between $LC_{50}$s were observed within a given species or stock for time intervals ≥ 48 h (Table 3 and Fig. 1). Additionally, all regression functions yielding ILL estimates had slopes $\le -0.5$ and $\le -0.3$ for Zn and Cu ($p < 0.05$), respectively, and the slopes were approximately parallel to the time axis. This suggests that the $LC_{50}$ values provided good predictors of the ILLs (Table 3). On the basis of $LC_{50}$s ≥48 h differences ($\alpha = 0.05$) and ILL estimates, the relative tolerance of the three species or stocks was hatchery rainbow trout > hatchery brown trout = Clark Fork brown trout.

The determined order of toxicity contributed by each individual metal to the 96-h $LC_{50}$ for rainbow trout exposed to the metals mixture was Cu > Zn > Cd > Pb, with Cu contributing 79.8%, Zn contributing 16.8%, and Cd contributing the remaining 3.4%. We were unable to complete a similar analysis for brown trout because $LC_{50}$ values for individual metals were not available in sufficient detail to match the water-quality conditions (e.g., hardness, pH) to the present study.

### Resistance to metals: estimates of mean time to death

Survival in both the control and metals-acclimated fish was 100% for both hatchery and Clark Fork brown trout and 98% for hatchery rainbow trout throughout the entire acclimation period. Acclimation to the metals mixture increased

**Table 3.** Estimates of 48-h $LC_{50}$ values (95% confidence limits in parentheses), ILL (slope of regression in parentheses, $p < 0.05$), and LTT for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout exposed to dilutions of a mixture of Zn, Cu, Pb, and Cd.

| Parameter | Species or stock | Zinc | Copper |
|---|---|---|---|
| 48-h $LC_{50}$ (μg/L) | Hatchery brown | 164.4*a* (146.4, 184.6) | 102.7*a* (91.9, 114.7) |
| | Clark Fork brown | 170.4*a* (151.3, 191.9) | 106.3*a* (94.8, 119.1) |
| | Hatchery rainbow | 239.7*b* (215.1, 267.0) | 148.0*b* (133.0, 164.7) |
| ILL (μg/L) | Hatchery brown | 159.0 (−0.30) | 96.5 (−0.18) |
| | Clark Fork brown | 166.1 (−0.50) | 103.8 (−0.30) |
| | Hatchery rainbow | 240.8 (−0.45) | 148.9 (−0.28) |
| LTT (h) | Hatchery brown | 32.8 | 32.3 |
| | Clark Fork brown | 31.7 | 31.4 |
| | Hatchery rainbow | 31.2 | 30.9 |

**Note**: $LC_{50}$ values were based on the measured Zn or Cu concentrations (μg/L) and the $LC_{50}$ calculations made using the Spearman–Karber method (Hamilton et al. 1977). For comparisons among species or stocks, $LC_{50}$ values followed by the same letter are not significantly different, on the basis of comparison of 95% confidence limits ($\alpha = 0.05$) with overall protection of $(0.95)^3 = 0.86$.

survival during subsequent challenge exposures in all species or stocks. Cumulative survival plots (challenges I and II, Figs. 2A and 2B) show similar survival rates for the naive brown and rainbow trout, and the fish that were acclimated to metals showed a marked increase in survival times compared with the naive fish. The increase in survival time was substantially greater for both hatchery and Clark Fork brown trout after 5 weeks of acclimation than after 3 weeks of acclimation. Furthermore, brown trout developed a substantially higher degree of resistance than did rainbow trout. Finally, the apparent effect of acclimation on survival time was lost for hatchery brown trout, but not for hatchery rainbow trout, following 2 weeks of exposure to control water (challenge III, Fig. 2C).

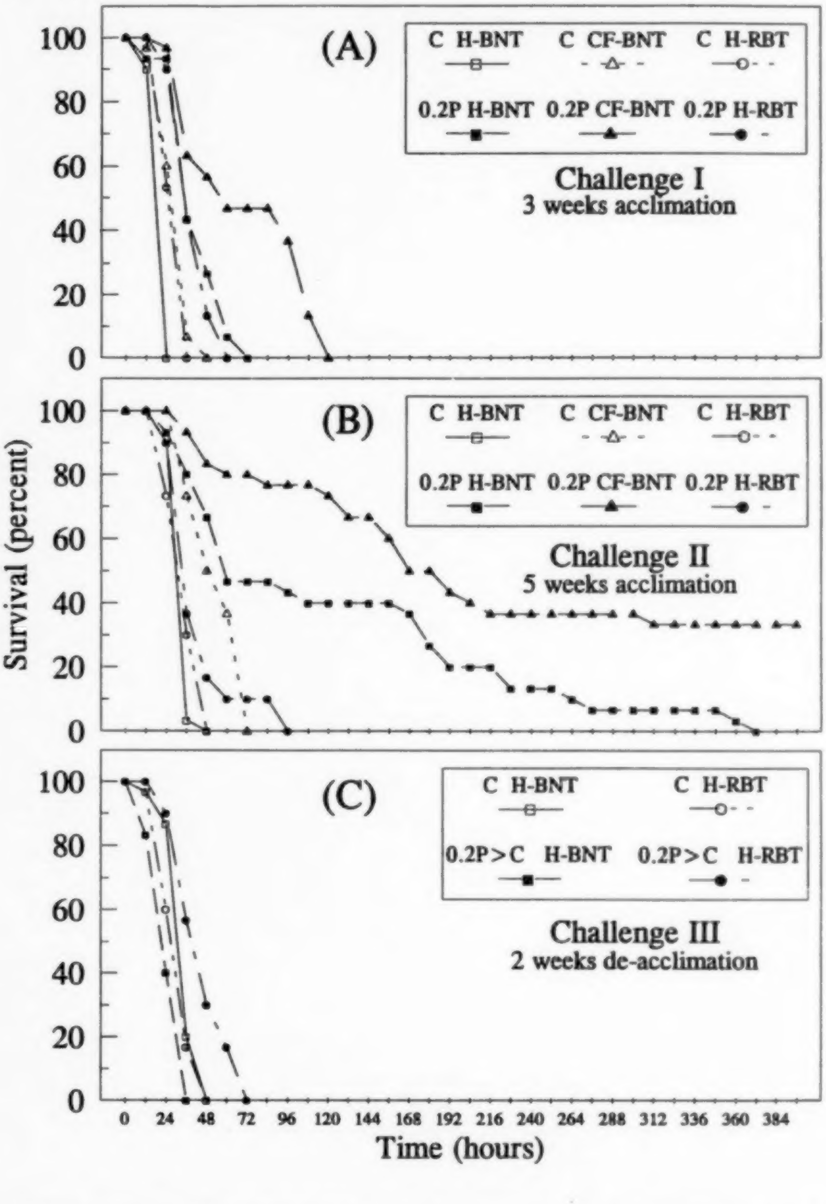
These results are supported by comparing acclimation effects and differences among species as demonstrated by the ANOVA and a priori *t* test results for mean TTD. For all three challenges, the species × acclimation interaction was significant ($p < 0.0001$), and therefore main effects in the overall ANOVA are not easily interpreted. Rather, a more detailed comparison was made between species on the basis of a priori *t* tests (Table 4). In challenge I (following 3 weeks of acclimation), mean TTD in controls was significantly longer for Clark Fork brown trout and hatchery rainbow trout than for hatchery brown trout, and Clark Fork brown trout also had a significantly longer mean TTD than either hatchery brown or hatchery rainbow trout when acclimated to the metals ($p < 0.01$; Table 4). In challenge II (following 5 weeks of acclimation), mean TTD in controls was significantly longer for Clark Fork brown trout than for either the hatchery brown or rainbow trout, and both the hatchery and Clark Fork brown trout had a significantly longer mean TTD than hatchery rainbow trout when acclimated to the metals ($p < 0.01$; Table 4). Both stocks of brown trout demonstrated significantly longer mean TTD post acclimation than similarly acclimated rainbow trout (Table 4 and Fig. 3). However, in the ANOVA for challenge II the covariate weight was significant ($p < 0.05$) and was included to adjust the means in the pairwise comparisons of species or stocks. Therefore, the differences in mean TTD between species may be confounded by the effects of fish size. In challenge III, after 2 weeks of de-acclimation, hatchery rainbow trout had a significantly longer mean TTD than hatchery brown trout in the de-acclimated groups ($p < 0.01$; Table 4).

Following 3 and 5 weeks of acclimation, each species or stock had a significantly longer mean TTD ($p < 0.01$, during the respective challenges) in the 0.2P acclimated groups than the corresponding controls (i.e., relative mean TTD was significantly positive; Fig. 3). The relative mean TTD increased for both Clark Fork and hatchery brown trout after 5 weeks of metals acclimation compared with the 3 weeks of metals acclimation, yet the effect was markedly greater in Clark Fork brown trout than in hatchery brown trout (Fig. 3). Again, this difference in relative mean TTD between hatchery and Clark Fork brown trout may, in part, be attributed to the difference in size of fish between the two stocks. Following 2 weeks of de-acclimation, hatchery rainbow trout initially acclimated to the metals but when they were placed in control water they still had a significantly longer mean TTD than the control. However, the initially acclimated hatchery brown trout had a significantly shorter mean TTD than the control ($p < 0.01$; Fig. 3).

### Acclimation effects on metallothionein, metal residues, and growth

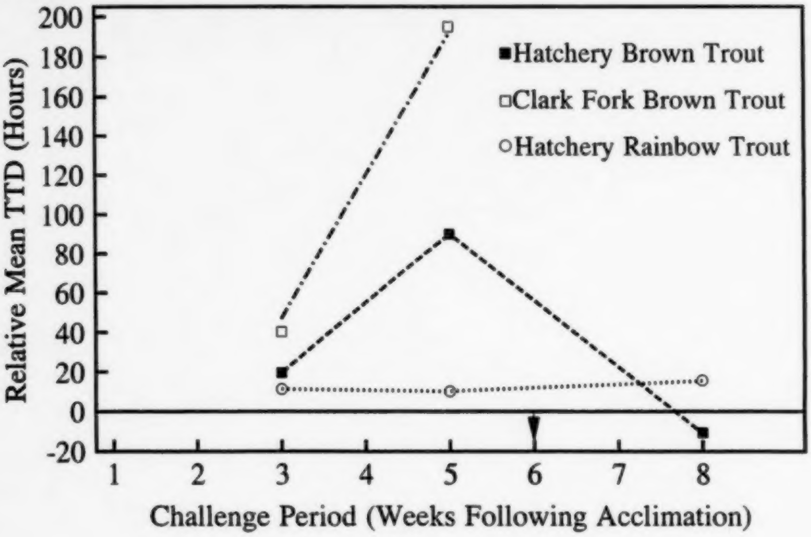
MT concentrations in liver were significantly higher in metals-acclimated fish than in controls for both hatchery brown and rainbow trout, but not in Clark Fork brown trout (Fig. 4). Overall, however, the main effect of acclimation on MT concentration was significant for each of the three species or stocks (hatchery brown trout, $F_{2,55} = 54.55$, $p < 0.001$; Clark Fork brown trout, $F_{2,46} = 7.16$, $p < 0.005$; hatchery rainbow trout, $F_{2,55} = 5.01$, $p < 0.05$). The interactions of acclimation and time were not significant, nor were the main effects of time for any species or stock. Pairwise comparisons revealed significantly higher mean MT concentrations (*i*) in the acclimated fish compared with

**Fig. 2.** Cumulative percent survival following acclimation to metals during each challenge period, where brown and rainbow trout were challenged with a 1P exposure. Challenges (A) I and (B) II followed 3 and 5 weeks acclimation, respectively, to low levels of metals, 0.2P, and control water, C, for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout. (C) Challenge III followed 2 weeks de-acclimation in control water for hatchery brown and rainbow trout. Nominal challenge concentrations for the 1P challenge exposure were 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd.



either control or de-acclimated fish, which were not different, for hatchery brown trout (Fig. 4A); (*ii*) in the de-acclimated fish compared with either control or acclimated fish, which were not different, for Clark Fork brown trout (Fig. 4B); and (*iii*) in the acclimated fish compared with control fish (but the de-acclimated fish were not different from either acclimated or control fish) for hatchery rainbow trout (Fig. 4C).

**Fig. 3.** Relative mean time to death (TTD, h) from challenges I (3 weeks acclimation), II (5 weeks acclimation), and III (2 weeks de-acclimation, initially indicated by arrow) for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout. Nominal challenge concentrations for the 1P challenge exposures were 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. For each challenge period and each species or stock, relative mean TTD values represent the difference between acclimation mean TTD and control mean TTD. All differences between the acclimation and control means were significant ($p < 0.011$ for challenges I and II, and $p < 0.025$, also see Table 4).



The matrix of partial correlation coefficients for MT and all metal residue concentrations in the livers of each species or stock identified Cu as the only metal significantly ($t = 5.35$, $p < 0.001$) correlated with MT. The subsequent tests for correlation between Cu and MT showed significant ($p \leq 0.001$) positive associations for each species or stock, with each brown trout stock $R^2 \geq 0.60$ (Figs. 4A and 4B) and hatchery rainbow trout $R^2 = 0.42$ (Fig. 4C). Among the four metals measured in the liver, the average concentrations were highest for Cu (Table 5). Unlike the concentrations of Cu, Zn, or Cd, the Pb concentrations in the de-acclimated fish were lower than in either the control or acclimated fish, especially for Clark Fork brown trout and hatchery rainbow trout. No apparent trend between acclimation groups emerged for residue concentrations of Zn or Cd.

Differences in weight between controls and metals-acclimated fish were evident in hatchery and Clark Fork brown trout, but not hatchery rainbow trout (Figs. 5A–C). The effect of acclimation and time on weight was significant in hatchery brown trout (acclimation, $F_{1,174} = 42.51$, $p < 0.001$; time, $F_{2,174} = 27.24$, $p < 0.001$) and in Clark Fork brown trout (acclimation, $F_{1,118} = 22.45$, $p < 0.001$; time, $F_{1,118} = 36.33$, $p < 0.001$), but only the effect of time was significant in hatchery rainbow trout ($F_{2,173} = 36.40$, $p < 0.001$). The interaction effect was not significant for any species or stock. At each time interval throughout the acclimation and de-acclimation periods, pairwise comparisons revealed significant differences in weights between controls and acclimated or de-acclimated groups for hatchery brown trout (Fig. 5A). Significant difference in weights between control and acclimated groups was found at the 5-week interval for Clark Fork brown trout (Fig. 5B). No significant differences in weight between controls and acclimated groups were found for hatchery rainbow trout (Fig. 5C).

No differences in food consumption or feeding behavior were apparent between acclimated and control fish or between species or stocks. Daily observations revealed that both acclimated and control fish of each species or stock consumed approximately 90–100% of their daily ration within an equivalent time period. Thus, food consumption or feeding behavior was not considered to be a potential variable in determining differences in growth among exposure groups or among species or stocks.

## Discussion

Survival of both brown and rainbow trout was reduced when fish were exposed to a metals mixture containing elevated concentrations of Zn, Cu, Pb, and Cd. Previously published laboratory and field studies have demonstrated adverse effects of metals such as Zn, Cu, Pb, and Cd on survival of salmonids and other freshwater organisms (Nehring and Goettl 1974; Finlayson and Verrue 1982; Roch and McCarter 1984*a*; Todd et al. 1984; Moore et al. 1991). On the basis of estimates of the percentage of toxicity contributed by each metal in the Clark Fork metals mixture, Cu and Zn contributed more than 96% of the toxicity of the mixture, and Cu dominated that percentage. Previous studies have demonstrated similar toxicity contributions using metals mixtures with Zn:Cu:Cd ratios comparable to the ratios used in the present study (e.g., Rehwoldt et al. 1971; Finlayson and Verrue 1982).

Additionally, acclimation to the metals mixture at concentrations typical of base flow conditions in the Clark Fork River increased the resistance of all three species or stocks during a challenge exposure to the metals mixture at Zn and Cu concentrations slightly above the ILL determined for rainbow trout. This was demonstrated by increases in mean TTD for the metals-acclimated fish. In both hatchery and Clark Fork brown trout, this acclimation effect on resistance to the metals challenge was dramatically greater after 5 weeks of metals acclimation than after 3 weeks of acclimation (e.g., mean TTD for Clark Fork brown trout increased from 26 or 50 h in control groups, to 66 h after 3 weeks of metals acclimation, and to 245 h after 5 weeks of metals acclimation; see Table 4). Moreover, the increase in resistance was greater for hatchery and Clark Fork brown trout than for hatchery rainbow trout after 5 weeks of metals acclimation, although this difference may have been attributable in part to differences in fish size between the two stocks. The results of the ANOVA for challenge II revealed that differences in weight were likely to have contributed to the differences in mean TTD between the three species or stocks. In spite of the dramatic acclimation response in brown trout, the increase in resistance was completely lost in hatchery brown trout after 2 weeks of de-acclimation; in fact, the hatchery brown trout seemed sensitized to the challenge-metals mixture after de-acclimation, as demonstrated by a negative value for relative mean TTD (Fig. 3). Moreover, in contrast to the brown trout, which were vulnerable to the metals challenge after de-acclimation, the rainbow trout retained their relatively slight resistance even after 2 weeks of de-acclimation (Fig. 3).

Many published studies have demonstrated acclimation to metals by fish, although in most of these studies single-metal exposures were used. For instance, increased resistance (e.g., increased LT$_{50}$s) or increased tolerance (e.g., increased LC$_{50}$s) to acutely lethal Zn concentrations has been demonstrated after pre-exposure to sublethal Zn concentrations in rainbow trout embryos (Sinley et al. 1974), rainbow trout fry (Bradley et al. 1985), rainbow trout juveniles (Lloyd 1960; Anadu et al. 1989), sockeye salmon alevins (Chapman 1978), and flagfish larvae (Spehar 1976; Spehar et al. 1978). Similar acclimation-induced increases in resistance or tolerance have been reported for various fish species pre-exposed to sublethal concentrations and then challenged with lethal concentrations of Cu (Dixon and Sprague 1981*a*), Cd (Pascoe and Beattie 1979; Duncan and Klaverkamp 1983; Benson and Birge 1985), and aluminum (McDonald et al. 1991; Mueller et al. 1991; Wilson et al. 1994*a*, 1994*b*). In studies using mixtures of metals, rainbow trout pre-exposed to a sublethal mixture of Zn, Cu, and Cd showed increased resistance or tolerance of a subsequent lethal challenge to the mixture (Roch and McCarter 1986, 1984*a*).

Increased tolerance and (or) resistance to metals has been explained by detoxification of metals by induced branchial MT (Hogstrand and Haux 1991). Our results suggest that hepatic MT is positively interrelated with Cu exposure and accumulation; this relationship may offer brown trout, and to a lesser extent rainbow trout, some protection against metal toxicity. These results are consistent with those of Dixon and Sprague (1981*b*) and McCarter et al. (1982), who suggested that Cu-induced de novo synthesis of hepatic MT was dependent on the level of Cu exposure, and that the binding of Cu by MT serves as a protective mechanism against toxicity.

**Table 4.** Mean and standard deviation (SD) for time to death (TTD, h) from challenge I (3 weeks acclimation), challenge II (5 weeks acclimation), and challenge III (2 weeks de-acclimation) for each combination of the two factors, where the first factor, species or stock, contains either three or two levels (hatchery brown trout, H-BNT; Clark Fork brown trout, CF-BNT; and hatchery rainbow trout, H-RBT) and the second factor, acclimation, contains two levels (C = control water, and 0.2P = metals acclimation or 0.2P→C = 2 weeks de-acclimation in control water following 6 weeks in 0.2P treatment).

| Species or stock | Acclimation condition | *n* | TTD (h) Mean | TTD (h) SD |
|---|---|---|---|---|
| **Challenge I (3 weeks acclimation)** | | | | |
| H-BNT | C | 30 | 18.73*a* | 4.19 |
| CF-BNT | C | 30 | 26.00*b* | 7.06 |
| H-RBT | C | 30 | 25.20*b* | 5.49 |
| H-BNT | 0.2P | 30 | 38.40*c* | 14.04 |
| CF-BNT | 0.2P | 30 | 66.40*d* | 36.40 |
| H-RBT | 0.2P | 30 | 36.60*c* | 9.13 |
| **Challenge II (5 weeks acclimation)** | | | | |
| H-BNT | C | 30 | 30.00*a* | 4.39 |
| CF-BNT | C | 30 | 49.60*b* | 16.20 |
| H-RBT | C | 30 | 28.60*a* | 8.62 |
| H-BNT | 0.2P | 30 | 120.07*c* | 102.78 |
| CF-BNT | 0.2P | 30 | 244.60*c* | 179.76 |
| H-RBT | 0.2P | 30 | 38.80*d* | 18.34 |
| **Challenge III (2 weeks de-acclimation)** | | | | |
| H-BNT | C | 30 | 30.87*a* | 7.22 |
| H-RBT | C | 30 | 25.87*a* | 8.85 |
| H-BNT | 0.2P→C | 30 | 20.20*b* | 7.57 |
| H-RBT | 0.2P→C | 30 | 41.60*c* | 15.25 |

**Note**: Nominal challenge concentrations for the 1P challenge exposures were 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. For each challenge period, mean values followed by the same letter are not significantly different on the basis of a priori *t* test results. Probability values for tests of equal mean TTD were judged to be significant at the conservative Bonferonni adjusted value (Manly 1991) of $\alpha = 0.10/9 = 0.0111$ for challenges I and II, and $\alpha = 0.10/4 = 0.025$ for challenge III.

In a field study of physiological impairment in resident brown trout from the upper Clark Fork River, significantly elevated MT was found in livers of fish from the Clark Fork River relative to uncontaminated reference sites (Farag et al. 1995). This field study also demonstrated elevated concentrations of Cu (as well as As and Cd; Zn was not

**Fig. 4.** Hepatic metallothionein and Cu concentrations (µg/g) in (A) hatchery brown trout, (B) Clark Fork brown trout, and (C) hatchery rainbow trout exposed to either control water (represented as open bar and ○), acclimation conditions (represented as cross-hatched bar and +; 0.2P metals concentrations, see text), or de-acclimation conditions (represented as single hatched bar and ▲; 0.2P→C, see text). For each species or stock, (left panel, I) mean values (±1 SEM) shown with the same letter superscript are not significantly different ($p < 0.05$). For each species or stock, (right panel, II) copper and metallothionein levels were significantly correlated ($p \leq 0.001$, $R^2$ = correlation coefficient). Note that the coordinates for two samples are not depicted in the plot (panel II) for the Clark Fork brown trout (B) de-acclimated fish, ▲, owing to the scales used; these two samples had Cu concentrations of 1106.5 and 1427.9 and corresponding MT concentrations of 969.3 and 615.8. Fish sacrificed for analyses were randomly selected at 0, 10, and 20 days into the acclimation and at 14 days following de-acclimation from the acclimation and control tanks.

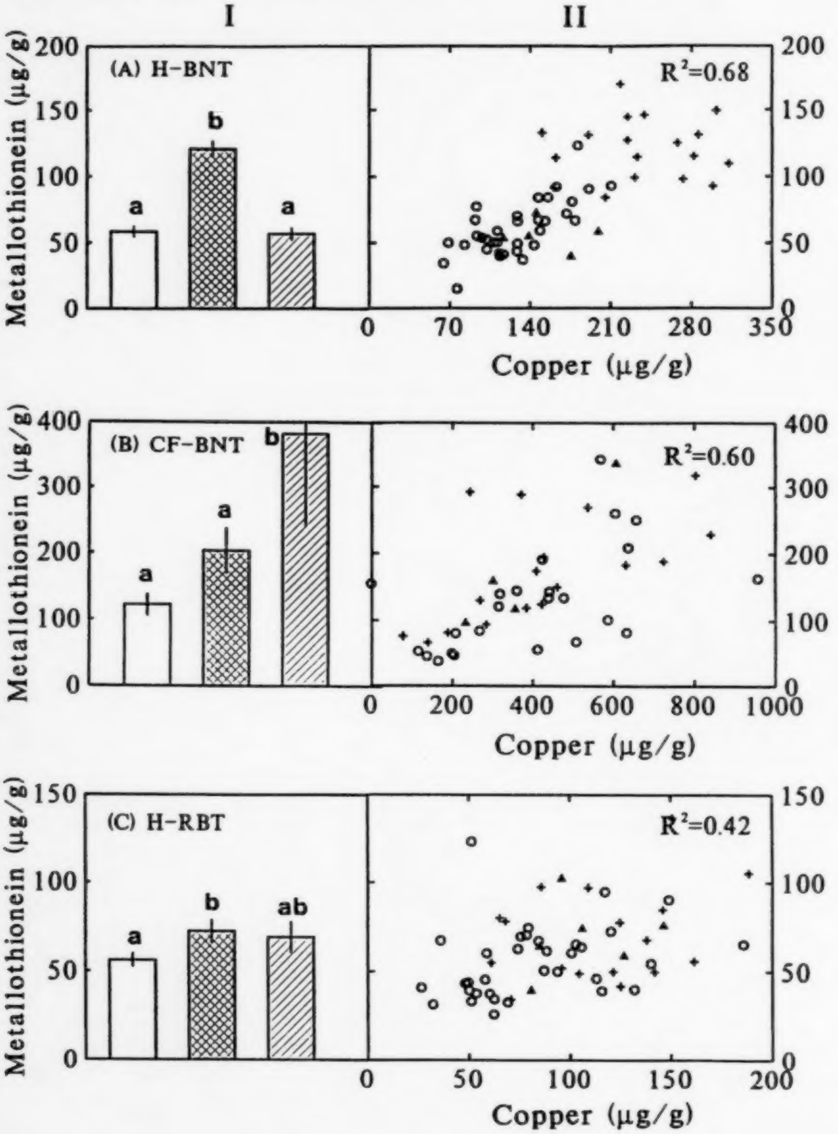


**Table 5.** Measured metal concentrations in liver tissue (µg/g, wet weight) from fish exposed to either control water (C; *n* = 34), acclimation water (0.2P, metals acclimation; *n* = 18), or de-acclimation water (0.2P→C, 2 weeks de-acclimation in control water following 6 weeks in 0.2P treatment; *n* = 6) during the acclimation experiments.

| Species or stock | Exposure | Zn | Cu | Pb | Cd |
|---|---|---|---|---|---|
| Hatchery brown | C | 3.95±1.11 | 129.01±36.75 | 0.41±0.27 | 0.13±0.11 |
| | 0.2P | 5.21±2.07 | 236.86±50.33 | 0.37±0.38 | 0.32±0.28 |
| | 0.2P→C | 3.90±1.54 | 190.43±90.80 | 0.21±0.12 | 0.22±0.15 |
| Clark Fork brown | C[a] | 3.47±1.43 | 403.43±217.71 | 0.26±0.31 | 0.22±0.13 |
| | 0.2P | 3.96±0.79 | 413.26±221.47 | 0.24±0.24 | 0.36±0.27 |
| | 0.2P→C | 4.36±1.52 | 671.39±489.24 | 0.06±0.09 | 0.83±0.50 |
| Hatchery rainbow | C | 3.77±1.45 | 82.88±36.51 | 0.36±0.30 | 0.16±0.22 |
| | 0.2P | 4.28±1.01 | 120.28±41.98 | 0.35±0.17 | 0.13±0.13 |
| | 0.2P→C | 3.56±0.42 | 106.89±25.49 | 0.03±0.03 | 0.23±0.08 |

**Note**: Values are given as the mean ± SD. Acclimation exposure water represents base flow concentrations (µg/L) of metals in the Clark Fork River, where metals concentrations for the 0.2P are 46 µg/L Zn, 24 µg/L Cu, 0.64 µg/L Pb, and 0.40 µg/L Cd. Average Zn, Cu, Pb, and Cd (µg/g) concentrations were determined by AAS. Fish sacrificed for analyses were randomly selected at 0, 10, and 20 days into the acclimation and at 14 days following de-acclimation from the acclimation and control tanks.

[a] *n* = 25.

measured) in several tissues sampled, including liver. Residue concentrations of Cu in liver were comparable in our laboratory-exposed brown trout and brown trout collected in the field by Farag et al. (1995). As in our study where similarities were shown between laboratory-exposed and control fish with respect to Pb residue concentrations in the liver, this field study also showed no difference between fish from uncontaminated reference sites and fish from the Clark Fork River. However, fish collected from a field site downstream of a contaminated site had the lowest Pb residues compared with fish from either the contaminated or reference sites (as did our de-acclimated fish compared with acclimated or control fish).

Although fish in our study were exposed to about twice as much Zn as Cu, Zn did not accumulate in the liver. These results are consistent with the rapid elimination of Zn from fish liver, whereas the liver is the main target for Cu accumulation (Hogstrand and Haux 1991; Hogstrand and Wood 1994). Roch and McCarter (1984*a*) also found MT to be predominantly associated with Cu when rainbow trout were exposed to a metals mixture containing 20 times more Zn than Cu. In our study, the binding of Cu by MT would likely offer the greatest protection for fish against the Clark Fork metals mixture because (*i*) Cu contributed the most to the toxicity of the mixture, (*ii*) Cu is in high concentration relative to Pb and Cd, and (*iii*) Cu is considerably more toxic to fish than Zn.

Physiological acclimation, including the production of MT, entails a metabolic cost. Our study documented weight reduction in brown trout that had increased mean MT concentrations. In contrast, hatchery rainbow trout did not show reduced growth, but were less able to acclimate to the metals. We did not observe any differences in food consumption or feeding behavior among the acclimated or control species or stocks that would attribute differences in growth to altered feed intake.

Previous studies on brown trout (Sadler and Lynam 1987, 1988; Reader et al. 1988), brook trout (Mount et al. 1988*a*, 1988*b*; Ingersoll et al. 1990*a*, 1990*b*), and rainbow trout (Wilson and Wood 1992; Wilson et al. 1994*a*, 1994*b*) have demonstrated reduced growth in response to aluminum exposure. Other direct measures of acclimation costs for juvenile rainbow trout acclimated to aluminum include reduced growth and reduced swimming capacity, related to a damage–repair phenomenon involving physiological, biochemical, and structural changes at the gills (Wilson et al. 1994*a*).

Reduced long-term growth also has been documented during sublethal exposures resulting in acclimation to Zn and Cu mixtures (Finlayson and Verrue 1980) and to Zn, Cu, and Cd mixtures (Roch and McCarter 1984*b*, 1986). In these studies, reduced growth was considered to be a sensitive measure of the deleterious effect of these metals mixtures. During sublethal exposures that resulted in acclimation to individual metals, reduced growth also has been documented for Zn (Hobson and Birge 1989) and Cu (Dixon and Sprague 1981*a*; Collvin 1984; Sprague 1985). Reduced growth associated with acclimation to metals such as Zn, Cu, and Cd is routinely considered to be the cost of acclimation and is often tied to specific structural (e.g., mucous cell proliferation; Wilson et al. 1994*a*), physiological (e.g., respiratory and ionoregulatory status; Collvin 1984; Wilson et al. 1994*b*), or biochemical (e.g., MT induction; Sprague 1985; Hobson and Birge 1989) changes that constitute the actual mechanisms of acclimation.

Overall, the results of our studies indicate that, on the basis of $LC_{50}$, ILL, and mean TTD determinations, the relative sensitivity of naive brown and rainbow trout to the metals mixture varies with the survival response measured (tolerance vs. resistance). However, when fish were acclimated to a sublethal metals mixture, brown trout were more resistant than rainbow trout to subsequent lethal chal-

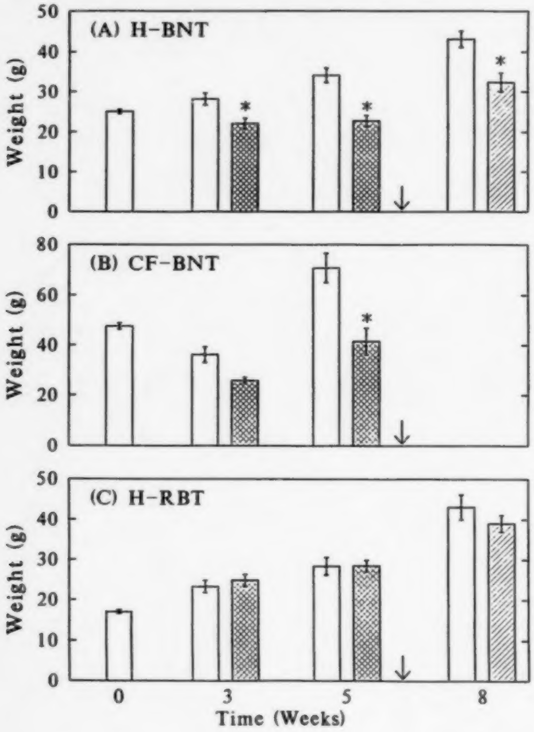


**Fig. 5.** Mean (±1 SEM; $n = 30$) weight of (A) hatchery brown trout (H-BNT), (B) Clark Fork brown trout (CF-BNT), and (C) hatchery rainbow trout (H-RBT) during acclimation experiments where fish were exposed to either control water (represented as open bar), acclimation conditions (represented as cross-hatched bar; 0.2P concentrations, see text), or de-acclimation conditions (represented as single hatched bar; $0.2P \rightarrow C$, initial de-acclimation time indicated by arrow). For each species or stock and time during acclimation, significant differences ($p < 0.05$) between mean values for control and acclimated or de-acclimated weights are indicated by asterisks.

lenges. This result is particularly interesting in view of the results of our companion study of pulse-induced mortality (Marr et al. 1995) in which rainbow trout were more tolerant than brown trout when metals concentrations only were increased. However, when metals concentrations were increased in conjunction with reductions in hardness and pH, rainbow trout were less tolerant than brown trout. In other words, in both studies (pulse exposure, acclimation), rainbow trout appeared to be either less or similarly sensitive to the base (acute) stress of metals, yet demonstrated increased sensitivity to complex stressors. We observe from these results that following pre-exposure, brown trout demonstrate greater physiological resiliency to metals toxicity than rainbow trout. This observation is consistent with the apparent relative sensitivity of rainbow trout to other complex environmental and biological stressors (e.g., temperature, dissolved oxygen content, competition) and may reflect evolutionary differentiation between brown and rainbow trout.

Finally, the results of this study confirm that although trout demonstrate an ability to acclimate to sublethal metals exposure, this acclimation entails a physiological cost that is apparent as reduced growth. Such growth reductions likely reduce the overall fitness of organisms in the wild and may ultimately contribute to the reduced viability in fish populations chronically exposed to sublethal concentrations of toxic metals.

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# Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana[1]

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**Abstract**: The avoidance response of brown trout (*Salmo trutta*) to mixtures of cadmium, copper, lead, and zinc was determined in water simulating the Clark Fork River, Montana. Effects of acidification on the avoidance response were also evaluated. Tests were conducted in a cylindrical chamber that received reference water at one end and metal-contaminated water at the other; a distinct boundary formed at the center where the chamber drained. A 1× mixture of the four metals (Cd, 1.1 μg/L; Cu, 12 μg/L; Pb, 3.2 μg/L; and Zn, 55 μg/L) that was representative of the ambient metals concentrations of the Clark Fork River resulted in avoidance by brown trout. Brown trout also avoided 0.5×, 2×, 4×, and 10× mixtures but not a 0.1× mixture. A reduction in pH from 8.0 to either 7.0, 6.0, or 5.0 resulted in significant avoidance. Avoidance reactions to metals, similar to those observed in our laboratory experiments, may contribute to the depression of brown trout populations in the Clark Fork River.

**Résumé** : Nous avons examiné la réaction d'évitement de la truite brune (*Salmo trutta*) à des mélanges de cadmium, de cuivre, de plomb et de zinc dans de l'eau simulant les conditions de la rivière Clark Fork, au Montana. Nous avons aussi évalué les effets de l'acidification sur la réaction d'évitement. Les essais ont eu lieu dans une chambre cylindrique qui recevait de l'eau témoin à une extrémité et de l'eau polluée par les métaux à l'autre; une limité nette se constituait au centre, où l'eau se vidangeait. Un mélange à 1× des quatre métaux (Cd, 1,1 μg/L; Cu, 12 μg/L; Pb, 3,2 μg/L; Zn, 55 μg/L), qui était représentatif des concentrations ambiantes de métaux de la rivière Clark Fork, a provoqué chez la truite brune une réaction d'évitement. La truite évitait aussi les mélanges à 0,5×, 2×, 4×, et 10×, mais pas un mélange à 0,1×. Une réduction du pH, de 8,0 à 7,0, 6,0 ou 5,0, provoquait une nette réaction d'évitement. Les réactions d'évitement en présence de métaux, comme celles qui ont été observées au cours de nos expériences en laboratoire, peuvent jouer un rôle dans l'affaiblissement des populations de truite brune de la rivière Clark Fork.

[Traduit par la Rédaction]

## Introduction

Metals contamination of the upper Clark Fork River basin (Fig. 1) in western Montana has been well documented (Johnson and Schmidt 1988; Luoma et al. 1989; Moore and Luoma 1990). The headwaters of the Clark Fork originate near the mining towns of Butte and Anaconda, which are known for their ore deposits of copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) (Miller 1973). Brown trout provide the main fishery in the upper Clark Fork River above Missoula, although the fish population is below the carrying capacity of the river. Metal contamination is considered the primary cause of reduced fish populations (Johnson and Schmidt 1988; Chapman 1993). Cu and Zn occur in the river at concentrations frequently exceeding the water-quality criteria established in the United States to protect freshwater aquatic life, and Cd and Pb concentrations occasionally exceed the criteria for those metals (U.S. Environmental Protection Agency 1987; U.S. Geological Survey 1989; Lambing 1991). The criteria for these metals are hardness dependent, but are at the following concentrations (μg/L) for the various metals at 100 mg/L hardness (as $CaCO_3$): Cd, 1.1; Cu, 12; Pb, 3.2; and Zn, 320.

Water quality criteria were formulated by the U.S. Environmental Protection Agency to protect survival, growth, and reproduction of aquatic life; however, behavioral avoidance of contaminants may be an additional cause of reduced fish populations (Sprague 1968; Folmar 1976;

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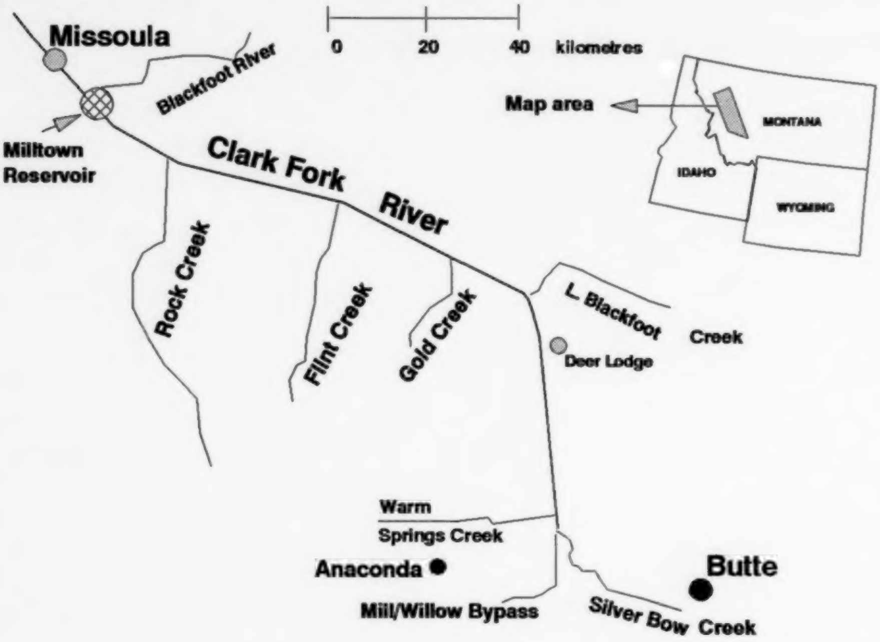
**Fig. 1.** Upper Clark Fork River basin in western Montana, U.S.A. Headwater tributaries of Silver Bow Creek, Mill Creek, and Willow Creek receive mining wastes from Butte and Anaconda.



Giattina et al. 1982; Giattina and Garton 1983; McNicol and Scherer 1991). Avoidance responses are particularly important because they may occur at concentrations lower than lethal thresholds (Little et al. 1993). Emigration resulting from avoidance of metals may contribute to depressed fish populations and may be partially responsible for the low brown trout populations in the Clark Fork River.

The avoidance response has been used as an indication of unfavorable water quality conditions, such as elevated carbon dioxide, or the presence of toxic substances, such as ammonia, copper, lead, and oil (Rice 1973; Beitinger and Freeman 1983; Gunn and Noakes 1986; Hartwell et al. 1987, 1989). Salmonids are particularly sensitive to metals, and they have been shown to avoid copper concentrations as low as 0.1 μg/L (Folmar 1976). Introduction of copper and zinc into a spawning tributary resulted in repulsion of ascending Atlantic salmon (Saunders and Sprague 1967). Prolonged exposure to metals has been shown to injure sensory tissue and impair or eliminate avoidance (Gardner and LaRoche 1973), allowing salmonids to be exposed to lethal concentrations.

The objective of our experiments was to investigate the hypothesis that brown trout prefer Clark Fork River water without metals to Clark Fork River water with metals. A second set of experiments tested the hypothesis that brown trout prefer unacidified Clark Fork River water to acidified Clark Fork River water. With the second hypothesis, we examined acidification alone and acidification in the presence of metals.

## Materials and methods

### Experimental fish

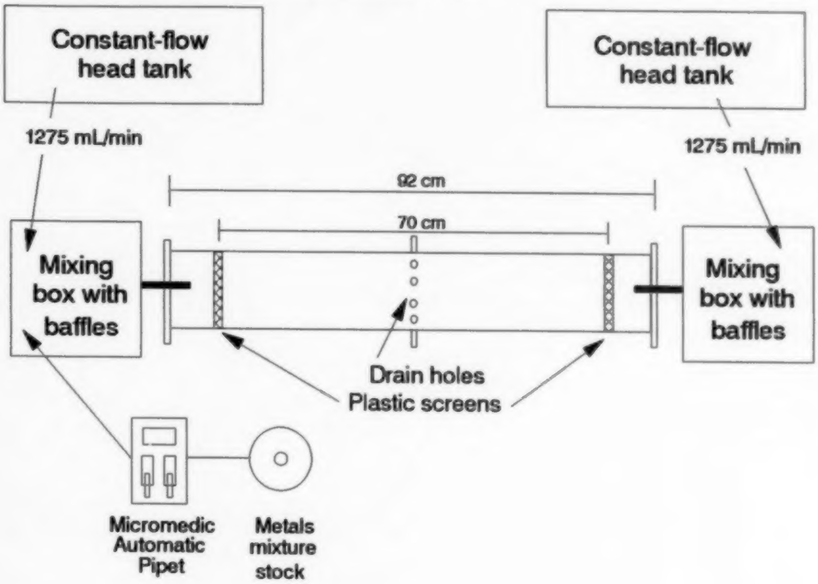
Brown trout were obtained as eggs from the Saratoga National Fish Hatchery in Wyoming and cultured at the Midwest Science Center (National Biological Service) Field Research Station, Jackson, Wyoming, under the following conditions: temperature, 10 ± 2°C; pH, 7.4; hardness, 160 mg/L (as $CaCO_3$); and alkalinity, 150 mg/L (as $CaCO_3$). Testing began 150 days after hatching and was concluded by 270 days. The length of experimental fish ranged from 55 to 85 mm, and fish were in good condition and free of obvious disease, injury, or distress.

### Reference and test water

Reference and test water simulated minimal conditions of hardness and alkalinity during spring in the upper Clark Fork River, when water quality would enhance metals toxicity (Lambing 1991). The Clark Fork River above Rock Creek has higher metals concentrations because the dilution affect of tributaries is minimal. Reference water used in this experiment simulated the Clark Fork River without metals and was used for acclimation and as the alternative choice for the fish to the test water. Test water was identical to reference water but was altered by the addition of metals or pH adjustment.

Reference and test water were prepared by mixing appropriate quantities of deionized water with well water to achieve an alkalinity of 100 mg/L (as $CaCO_3$), hardness

**Fig. 2.** Avoidance chamber. Water enters each end of chamber through the mixing box and exits at the central drain holes. Test end of chamber receives metals mixture and (or) acid by automatic pipet. Chamber diameter is 11 cm.



of 100 mg/L (as $CaCO_3$), and conductivity of 200 µS/cm. Reagent grade metals (VWR Scientific) were measured individually into a common stock container. The pH for each water was adjusted to 8.0 ± 0.25 using $H_2SO_4$.

### Chemical analyses of water

Test and reference water were prepared each day of testing and analyzed for hardness, alkalinity, and conductivity. The values for these parameters were within 10% of nominal. When pH was a variable in the test water, pH was measured in the reference and test water twice during each avoidance test with an Orion model EA 940 ion analyzer.

Metals analysis was performed on 100-mL filtered samples collected from test water after each replicate test and from reference water 12 times during testing. Water samples were filtered through a 0.3-µm filter, transferred to a precleaned 125-mL polyethylene bottle, and preserved by addition of 1 mL nitric acid. Dissolved Cd, Cu, and Pb were determined by graphite furnace atomic absorption spectrophotometry, and Zn was analyzed by flame atomic absorption spectrophotometry. Instrument detection limits for Cd, Cu, Pb, and Zn were 0.4, 1.2, 1.7, and 4.9 µg/L, respectively. All analyses were conducted at the University of Wyoming.

### Test apparatus

The avoidance chamber was a Plexiglas cylinder (11 cm diameter by 92 cm) similar to the design used by Sprague (1968) (Fig. 2). Water entered each end of the chamber from the mixing box at 1275 ± 100 mL/min, flowed towards the center, and exited through six drain holes. Plastic screens were placed 11 cm from each end of the chamber to create a 70-cm observation area. The underside of the chamber was covered with light blue contact paper to provide an even visual background. The chamber and test area were surrounded with black plastic to minimize external disturbance. An overhead video camera was installed 110 cm above the tank to monitor and record fish positions from a remote location.

Water of the desired quality and temperature was pumped to a 54-L glass constant-head tank at each end of the avoidance chamber (Fig. 2). Each head tank drained into a glass mixing box that had a series of baffles to aid in the mixing of water, metals solution, and acid prior to entering the avoidance chambers. Metals solution or acid ($H_2SO_4$) was metered into the mixing box with an automatic pipet (Micromedic) as needed to create the test water. Water flow through the mixing box was controlled by the constant head pressure and a glass valve.

Observations of the distribution of fluorescein dye within the chambers showed that a defined gradient was established 5 min after the introduction of the dye, and all coloration cleared the chamber within the 20-min rinse period between tests. The gradient remained within 5 cm of the center of the drain holes at all times and was quickly reestablished after fish moved through the gradient.

### Test procedure

Fish were acclimated to the temperature and hardness of the test water for at least 72 h prior to testing. Changes in temperature during acclimation did not exceed 1°C/day, and changes in hardness did not exceed 25%/day. Water temperature was maintained at 12 ± 1.0°C during testing, and the temperature differential between the test and reference

**Table 1.** Mean (SD) for measured concentrations (μg/L) of metals in filtered test water, $n = 6$.

| Test water | Cd | Cu | Pb | Zn |
|---|---|---|---|---|
| 0 | <0.4 | <1.2 | <1.7 | 9.7±4.5 |
| 0.1 | <0.4 | 1.9±1.2 | <1.7 | 14±2.3 |
| 0.5 | 0.6±0.1 | 6.5±0.3 | <1.7 | 32±4.5 |
| 1 | 1.3±0.5 | 11±0.6 | 4.4±0.4 | 51±2.5 |
| 2 | 2.1±0.1 | 22±0.8 | 9.0±0.8 | 105±7.2 |
| 4 | 4.2±0.1 | 50±1.9 | 17±0.7 | 213±32 |
| 10 | 11±0.4 | 115±4.0 | 37±2.9 | 470±33 |

**Note:** Values are given as the mean ± SD. The nominal concentrations of metals in test water at the 1× strength were as follows (in μg/L): Cd, 1.1; Cu, 12; Pb, 3.2; and Zn, 50.

ends of the avoidance chamber was never more than 0.4°C. Fish were not fed 12 h before testing.

Avoidance testing included two phases: a 20-min acclimation phase followed by a 30-min test phase. The acclimation phase was initiated by placing one fish in a randomly selected end of the chamber. The video recorder and on-screen timer were activated. In the test phase, the metering pipets were activated, and a contaminant gradient was created in a randomly selected end of the chamber. The first 10 min of the test phase was a latency period, when the contaminant gradient was established. Observations of the avoidance response were quantified during the final 20 min of the test phase and included the time spent in the test-water end of the chamber, the number of trips into the test-water end of the chamber, and mean trip time. One trip was defined as entrance into and exit out of the test end of the chamber. Fish were required to have a minimum of 5 trips during the 30-min test period. A minimal number of trips was needed to ensure that fish were experiencing the test and reference sides of the avoidance chamber. The 5-trip minimum was met 355 times out of 360 tests. After testing, the fish was removed from the chamber and length was measured.

There was a 20-min rinse period between tests during which reference water flowed into both ends of the chamber with no fish present. At this time, mixing boxes were rinsed with a HCl solution (pH 2.0) followed by three reference water rinses.

### Avoidance of different metals concentrations

Seven metals mixtures were tested: 0× (no metals) as the control, 0.1×, 0.5×, 1×, 2×, 4×, and 10×. The 1× metals mixture was representative of ambient concentrations measured in the Clark Fork River (Lambing 1991), where Cd ($CdCl_2$) was 1.1 μg/L, Cu ($CuCl_2$) was 12 μg/L, Pb ($PbCl_2$) was 3.2 μg/L, and Zn ($ZnCl_2$) was 50 μg/L. The additional treatments were tested to represent the variation of low to high metals concentrations that can exist in the Clark Fork River.

### Avoidance of acidity alone and acidity with metals

We determined if short-term increases in acidity (such as those known to be associated with storm events in the Clark Fork River) influence the avoidance response of brown trout to water with and without 1× metals. Four pH levels were evaluated: 5.0, 6.0, 7.0, and 8.0, with 8.0 as the control. Test water with these same four pH values was also evaluated in combination with 1× metals, where the control was pH 8.0 water with 1× metals.

Reducing pH with $H_2SO_4$ can result in an increase in $CO_2$. However, pretest measurements of $CO_2$ in pH 5.0 test water indicated that the baffles in the mixing box aerated the test water sufficiently to remove any $CO_2$ produced during acidification.

### Experimental design and statistics

Two avoidance chambers were used; therefore, we were unable to test all treatments simultaneously. However, a balanced incomplete block design was used to eliminate time as an experimental variable (Cochran and Cox 1957). Two treatments were tested per day, and the treatment pairs were assigned to test days on a rotating schedule until six avoidance trials were performed for each treatment. Each treatment was compared with every other treatment equally often and with the same precision.

For each trial four separate avoidance tests were conducted on 1 day in one chamber for a total of 24 fish tested per treatment (4 tests per trial × 6 trials per treatment). The test end of the avoidance chamber was determined at random for the first observation; thereafter the test end was alternated for each succeeding observation. Placement of fish into the avoidance chamber was alternated between ends.

The three observations made from each avoidance test were transformed to natural logs to homogenize variance and normalize distributions. Analysis of variance and Tukey's least significant difference tests were performed on the transformed data to compare differences between treatment means ($P \leq 0.05$). Time spent in test water was also reported as a percentage.

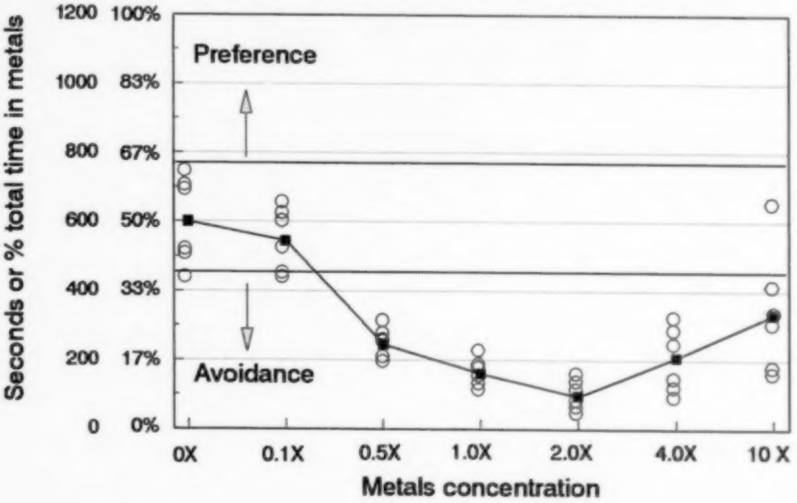
## Results

### Avoidance of different metals concentrations

Metals concentrations measured in test water were within 10% of the nominal concentration (Table 1) except for Pb concentrations, which were 30–40% higher than the nominal concentration in the 1×, 2×, and 4× test waters. In the 0.1× water, Cd and Pb were below the measurable detection limits; in the 0.5× test water, Cd and Pb were at the detection limits.

The amount of time in the test-water end of the avoidance chamber was a function of the number of trips into the test-water end and the length of time for each trip. If there were no preference or avoidance, the fish would be expected to spend equal time in the test and reference water (600 s, 50%). The response of brown trout to metals in water can be used to illustrate the measurement of the avoidance–preference response (Fig. 3). Avoidance was significant when total time in the test water was reduced below 445 s, or 37% of the total time. Conversely, preference would have occurred when total time in the test water was greater than 776 s, or 65% of the total time. Therefore, brown trout significantly avoided simulated Clark Fork River water when metals concentrations were 0.5× or greater.

**Fig. 3.** Avoidance–preference response of brown trout to seven metals concentrations (0×, 0.1×, 0.5×, 1×, 2×, 4×, and 10×) when tested against the 0× reference water during a 1200-s test. Parallel lines at 37 and 65% represent the 95% confidence interval for the response of the fish in the 0× reference water. Nominal concentrations (μg/L) of metals in the test water for the 1× strength: Cd, 1.1; Cu, 12; Pb, 3.2; and Zn, 50. ■, mean of 6 avoidance trials; ○, mean of the 4 avoidance tests per trial.



Percentage of total time in test water dropped from 50% for 0× test water to less than 20% for 0.5×, 1×, 2×, and 4× test waters (Table 2). The greatest avoidance was observed at 2× and 1×, when brown trout spent 8 and 13%, respectively, of their time in the test water.

### Avoidance of acidity and metals

During avoidance testing with increased acidity, pH values of 6.0, 7.0, and 8.0 were maintained at ±0.25 pH units; the pH of 5.0 was maintained at ±0.5 pH units. The mean measured metal concentration was within 10% of the nominal value for each test water (data not shown).

Brown trout significantly avoided simulated Clark Fork River water (0×) at pH values of 5.0, 6.0, and 7.0 (Table 3). Total time in test water dropped from 48% at pH 8.0, to less than 20% at pH 7.0, to less than 10% at pH 6.0 and 5.0. When 1× metals were presented in combination with pH 8.0, brown trout spent only 13% of their time in the test water, which is similar to the results observed in the 1× test water during avoidance testing of different metals concentrations (Table 2). The total time in test water of pH 5.0, 6.0, and 7.0 and 1× metals was less than 10%, but not significantly below the response observed in pH 8.0 with 1× metals. However, the number of trips into test water by fish at pH values of 5.0, 6.0, and 7.0 with 1× metals was significantly below the response of fish at pH 8.0 with 1× metals.

**Table 2.** Avoidance of metals by brown trout in test waters simulating the Clark Fork River.

| Test water (×) | Total time in test water (s) | Percent time in test water | Number of trips into test water | Mean trip duration in test water (s) |
|---|---|---|---|---|
| 0 | 601±132 | 50±11 | 61±2.6 | 11±2.2 |
| 0.1 | 545±95 | 45±8.0 | 58±8.1 | 10±2.9 |
| 0.5 | 245±34$^a$ | 20±2.8$^a$ | 48±6.4 | 5.7±1.9$^a$ |
| 1 | 161±33$^a$ | 13±2.8$^a$ | 46±9.6 | 3.7±0.4$^a$ |
| 2 | 96±45$^a$ | 8±3.7$^a$ | 31±6.8$^a$ | 3.0±0.5$^a$ |
| 4 | 209±87$^a$ | 17± 7.3$^a$ | 40±6.8$^a$ | 4.3±1.3$^a$ |
| 10 | 333±182$^a$ | 28±15$^a$ | 54±26 | 5.7±1.9$^a$ |

**Note**: Values are given as the mean ± SD. The nominal concentrations of metals in test water at the 1× strength were as follows (in μg/L): Cd, 1.1; Cu, 12; Pb, 3.2; and Zn, 50.

[a] Significantly different from 0× metals concentration ($P \leq 0.05$).

## Discussion

Brown trout avoided metals mixtures at concentrations similar to those avoided by other fish species for individual metals. The lowest Cu concentrations avoided by rainbow trout (*Oncorhynchus mykiss*) ranged from 0.1 to 7.3 μg/L (Folmar 1976; Giattina et al. 1982). Sprague (1964) found that young Atlantic salmon (*Salmo salar*) could detect and avoid 2.3 μg/L copper. Rainbow trout avoided 5.6 μg/L Zn (Sprague 1968), but Atlantic salmon had a Zn avoidance threshold of 53 μg/L (Sprague 1964). Reduced pH resulted in avoidance by brook trout (*Salvelinus fontinalis*) (Gunn and Noakes 1986), a finding also observed in our studies.

Sprague (1964) performed avoidance testing on a mixture of Cu and Zn and found that the combination of the two metals reduced the avoidance threshold below that for each metal tested individually. In combination, 0.4 μg/L Cu

**Table 3.** Avoidance response of brown trout to test water having four different pH values with and without 1× metals concentration.

| pH | Metals concentration (×) | Total time in test water (s) | Percent time in test water | Number of trips into test water | Mean trip duration in test water (s) |
|---|---|---|---|---|---|
| | | | **Effects of acidity only** | | |
| 8.0 | 0 | 582±96 | 48±8.0 | 57±20 | 14±7.1 |
| 7.0 | 0 | 202±32[a] | 17±2.6[a] | 43±3.6 | 4.6±0.9[a] |
| 6.0 | 0 | 81±11[a] | 6.8±0.9[a] | 29±2.5[a] | 2.8±0.4[a] |
| 5.0 | 0 | 84±14[a] | 7.0±1.2[a] | 29±5.0[a] | 3.0±0.5[a] |
| | | | **Effects of acidity and metals** | | |
| 8.0 | 1 | 157±82 | 13±6.8 | 45±12 | 3.5±1.1 |
| 7.0 | 1 | 72±23 | 6.0±1.9 | 31±5.6[b] | 2.4±0.4 |
| 6.0 | 1 | 113±106 | 9.4±8.8 | 21±3.9[b] | 4.8±4.8 |
| 5.0 | 1 | 63±21 | 5.3±1.8 | 25±6.3[b] | 2.4±0.38 |

**Note**: Values are given as the mean ± SD; $n = 6$, with each value based on four avoidance tests.

[a] Significantly different from value at pH 8.0 (acidity only tests).

[b] Significantly different from value at pH at 8.0 with 1× metals (acidity and metals tests).

and 6.1 μg/L Zn produced an avoidance reaction in Atlantic salmon that was 6–8 times lower than the concentration required to initiate avoidance from the individual thresholds of 2.3 μg/L Cu and 53 μg/L Zn. The lowest concentrations of Cu and Zn avoided in our experiment (0.5× treatment) were 6.5 μg/L Cu and 32 μg/L Zn, which are comparable to the values reported by Sprague (1964) for the individual metals.

Avoidance of Cd and Pb has not been studied as extensively as that of Cu and Zn in salmonids. McNicol and Scherer (1991) reported that lake whitefish (*Coregonus clupeaformis*) avoided Cd at a threshold of 0.2 μg/L. However, the avoidance response was only significant at less than 1 μg/L or greater than 8 μg/L. In more recent tests, lake whitefish showed a neutral response to Cd at 0.2, 1, and 5 μg/L (McNicol and Scherer 1993). Giattina and Garton (1983) reported an avoidance threshold of 26 μg/L Pb for rainbow trout.

In a study similar to ours (Hansen 1993), rainbow trout avoided lower concentrations of metals than we observed for brown trout. Giattina and Garton (1983) reported that the difference in avoidance response between species can be explained by inherent activity level. In closely monitored studies on feeding behavior, we measured slower response time and less activity in brown trout than in rainbow trout (Woodward et al. 1995). Therefore, the avoidance of metals by rainbow trout may be increased over that by brown trout by the greater activity level of rainbow trout.

At the highest metals concentrations tested (4× and 10×), there was a trend toward reduced avoidance compared with the responses at the 1× and 2× concentrations. At high Cu concentrations perceptive acuity may be impaired as a result of injury of sensory tissue (Gardner and LaRoche 1973; Hara et al. 1976). If brown trout were slow to initially avoid the 10× concentration in our experiments, injury to olfactory function may have affected the ability of some fish to detect and avoid the 10× metals.

In our experiment, the 1× and 0.5× mixtures contained Cd, Cu, Pb, and Zn that were at or below the water-quality criteria concentrations established by the U.S. Environmental Protection Agency for protection of freshwater aquatic life (U.S. Environmental Protection Agency 1987). Brown trout spent only 13% of their time in water containing the 1× mixture, and 20% of their time in water containing the 0.5× concentration. Therefore, at the water-quality criteria concentrations for these metals, we can expect fish to avoid conditions in the Clark Fork River.

The frequency at which water-quality criteria for Cd, Cu, Pb, and Zn have been exceeded in the Clark Fork River has been summarized (Lambing 1991; Kerr 1993). The criterion concentration increases with increased water hardness because Cd, Cu, Pb, and Zn tend to be more toxic at lower hardnesses. The actual criterion concentration for each metal is determined by an equation that weighs hardness and the toxicity of that metal. From 1970 to 1992, criteria were exceeded in the Clark Fork River from the Milltown reservoir to its headwaters in all reaches for all metals, although the Cu criteria were exceeded more frequently and more severely. The Cu criteria were exceeded in 22% of all samples collected between the Milltown reservoir and Warm Springs Creek, and in 100% of the samples from Silver Bow Creek.

The results of our avoidance experiments combined with metals monitoring data suggest that brown trout would avoid the Clark Fork River and Silver Bow Creek above the Milltown reservoir. Studies comparing numbers and biomass of brown trout in the upper Clark Fork River and Silver Bow Creek with reference sites support our findings (Chapman 1993). There is a complete absence of trout species in Silver Bow Creek. Reference sites contained an average of 36 times more juvenile trout and 11 times more adult trout than did Clark Fork River sites. Reference sites had low metals concentrations but were otherwise comparable to Clark Fork River sites on the basis of geology, habitat, flow rate, and water uses. Differences in trout populations between sites are most likely due to metals contamination. Therefore, we believe that the reduced trout population in the Clark Fork River and Silver Bow Creek is due in part to avoidance of metals contamination of surface water.

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# The physiological impairment of free-ranging brown trout exposed to metals in the Clark Fork River, Montana[1]

**Aïda M. Farag, Mark A. Stansbury, Christer Hogstrand, Elizabeth MacConnell, and Harold L. Bergman**

**Abstract**: Tissue metal accumulation and physiological responses were measured in brown trout (*Salmo trutta*) collected from two sites on the Clark Fork River, Montana, and two reference sites to determine the health status of the resident fish population. Concentrations of metals in tissues, products of lipid peroxidation, and microscopic Cu inclusions were significantly higher in fish collected from the upper Clark Fork River site than in fish from reference sites. Metallothionein concentrations were higher in the livers of fish from the upper Clark Fork River than in those of fish from reference sites. The health of the fish population in the upper Clark Fork River is probably impaired because of previously reported elevated concentrations of metals including As, Cd, Cu, Pb, and Zn in the surface water and aquatic invertebrates in the upper Clark Fork River. The high concentrations of metals in the surface waters and sediments of the Clark Fork River are typical of rivers in the intermountain western United States where discharges from mining activities have occurred since the late 1800s. Thus, our findings can be used as a guide for researchers throughout the intermountain western United States who may investigate similar field conditions.

**Résumé** : Nous avons mesuré l'accumulation de métaux dans les tissus et les réactions physiologiques chez des truites brunes (*Salmo trutta*) prélevées à deux sites de la rivière Clark Fork, au Montana, et à deux sites témoins afin de déterminer l'état de santé de la population résidante de poissons. Les concentrations de métaux dans les tissus, les produits de la peroxydation des lipides et les inclusions microscopiques de Cu étaient nettement plus élevés chez les poissons prélevés au site de la rivière Clark Fork d'amont que chez les poissons des sites témoins. Les concentrations de métallothionéine étaient plus élevées dans le foie des poissons du site de la rivière Clark Fork d'amont que chez les poissons des sites témoins. La santé de la population de poissons du tronçon d'amont de la rivière Clark Fork est probablement affaiblie à cause de concentrations élevées, déjà signalées, de métaux comme As, Cd, Cu, Pb et Zn dans l'eau de surface et chez les invertébrés aquatiques. Les fortes concentrations de métaux dans les eaux de surface et les sédiments de la rivière Clark Fork d'amont sont typiques des cours d'eau de la zone intramontagnarde de l'ouest des États-Unis qui subissent, depuis la fin du dix-neuvième siècle, les rejets des activités minières. Nos résultats peuvent donc guider les chercheurs qui, dans cette zone des États-Unis, voudraient faire des études sur des conditions de terrain similaires.

[Traduit par la Rédaction]

## Introduction

Surface water and sediments from the upper Clark Fork River contain elevated concentrations of metals including As, Cd, Cu, Pb, and Zn (U.S. Geological Survey 1989; Lambing 1991; Moore et al. 1991). However, measurements of pollutants in water and sediment alone are sometimes difficult to link to fish health because fish move in and out of polluted areas and because water quality fluctuates through time (Flemming and Trevors 1989). Additionally, water and sediment analyses do not account for toxicity caused by ingestion of contaminated food (Komarovsky and Polishchuk 1981).

The physiology of some invertebrate species renders them able to accumulate metals to high concentrations. For instance, mollusks and crustaceans contain hemocyanin, a blood pigment that contains Cu, and most invertebrates

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[1] This is part of a collective group of publications on the Clark Fork River, Montana.

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sequester metals in the carapace or exoskeleton (Timmermans 1993). Thus, the physiology of benthic invertebrates and their constant contact with contaminated sediments make them a potential dietary source of metals for fish in the Clark Fork River. Invertebrates in reaches of the Clark Fork River have high concentrations of metals when compared with animals from reference sites (Woodward et al. 1994, 1995; Farag et al. 1995). In particular, As, Cu, Cd, Pb, and Zn have been measured in concentrations of 2–12 times that of references sites (Woodward et al. 1994, 1995; Farag et al. 1994).

Physiological responses to exposure to metals and accumulation of metals in tissues can be used to supplement the monitoring of fish populations and water and sediment quality that is typically conducted in environmental hazard assessments of metal-contaminated aquatic environments. Moreover, physiological measurements and associated tissue metal residue concentrations can help in determining cause and effect relationships that might exist. Researchers may not determine that the health status of a fish population is compromised if tissue metal concentrations alone are high, but if those elevated tissue metal concentrations are associated with indications of physiological impairment (e.g., increased lipid peroxidation) in the same fish, it may be concluded that the health status of a fish population is impaired. Further associations among tissue metal accumulation(s), oxidative stress, and growth reduction would add more weight to a determination of fish health impairment. This weight of evidence approach uses all of the information gathered to determine the health status of a fish population. The objective of this study was to determine the degree of fish health impairment caused by chronic exposure of brown trout (*Salmo trutta*) to metals in the Clark Fork River. Adult brown trout were collected from the Clark Fork River and from two reference sites for determinations of metal residue accumulation and physiological parameters related to fish health.

## Methods

### Sample collection

During May 13–14, 1992, adult brown trout were collected by electrofishing from each of four sites (Fig. 1). Two of the sites were located on the Clark Fork River, an upstream site below Warm Springs ponds and a downstream site near Turah Bridge. These sites include high and low, respectively, concentrations of metals in water, sediments, and benthic macroinvertebrates. The third and fourth sites were reference sites located on the Big Hole River (adjacent drainage to the Clark Fork River) and Rock Creek (a tributary to the Clark Fork River). To characterize baseline physiology for statistical comparisons, we collected samples from two reference sites rather than one. Ten fish each were collected at Warm Springs ponds and Big Hole River; nine fish each were collected at Turah Bridge and Rock Creek.

In the field, the length and weight of each fish were measured. The fish were then examined using a standard necropsy procedure (Goede 1989) and blood, gill, liver, kidney, pyloric caeca, stomach, and large intestine were sampled. The contents of stomachs and large intestines were removed and these two tissues were rinsed. Sections of each tissue (and the whole spleen) from each fish were placed in Bouin's solution for subsequent histologic examination. The remaining portions of tissue and stomach contents were frozen immediately in liquid nitrogen and placed on dry ice for transport to the University of Wyoming Red Buttes Environmental Biology Laboratory where they were stored at −70°C. Each tissue was later ground in a mortar cooled with liquid nitrogen and divided into aliquants for tissue metal, lipid peroxidation, and metallothionein (MT) measurements. Five additional fish from the Big Hole River, Turah Bridge, and Warm Springs sites were collected and frozen for whole-body residue analyses.

### Metal residues in fish tissues

Gill, liver, kidney, and pyloric caeca tissues were lyophilized, digested with Ultrex nitric acid in a microwave oven, and taken up to a 50 mL final volume in a 5% $HNO_3$ matrix at the Department of Health and Environmental Science, Chemistry Laboratory Bureau, Helena, Montana. The samples were then transported to Butte, Montana, for chemical analyses by the Montana Bureau of Mines Analytical Laboratory. Fish tissues were analyzed for As, Cu, Cd, and Pb using inductively coupled plasma emission – mass spectroscopy (ICP-MS) on a Perkin-Elmer Elan 5000 (Norwalk, Conn.).

Analytical protocols defined by EPA method 200.8 (U.S. Environmental Protection Agency 1991) were followed with minor deviations. Recommendations of the manufacturer were followed in setting up the ICP-MS for analysis. Initial calibration verification, continuing calibration verification, low-level standard verification, and spike and duplicate measurements were used to verify the analytical method. Analytical duplicates and spikes were analyzed every 10 samples. No appropriate reference samples were commercially available for all of the matrices, hence we submitted duplicate tissues on a regular basis (in addition to bovine liver standards) and used them for verification.

Whole fish were partially thawed and homogenized in a stainless steel blender, and 2- to 3-g samples of the homogenate were lyophilized. The lyophilized whole fish, stomach, large intestine, and stomach contents were digested in 5 mL of 30% Ultrex $HNO_3$ and analyzed for As, Cu, Cd, and Pb by graphite furnace atomic absorption spectroscopy (Perkin-Elmer model 2380). Quality assurance and quality control were performed as above.

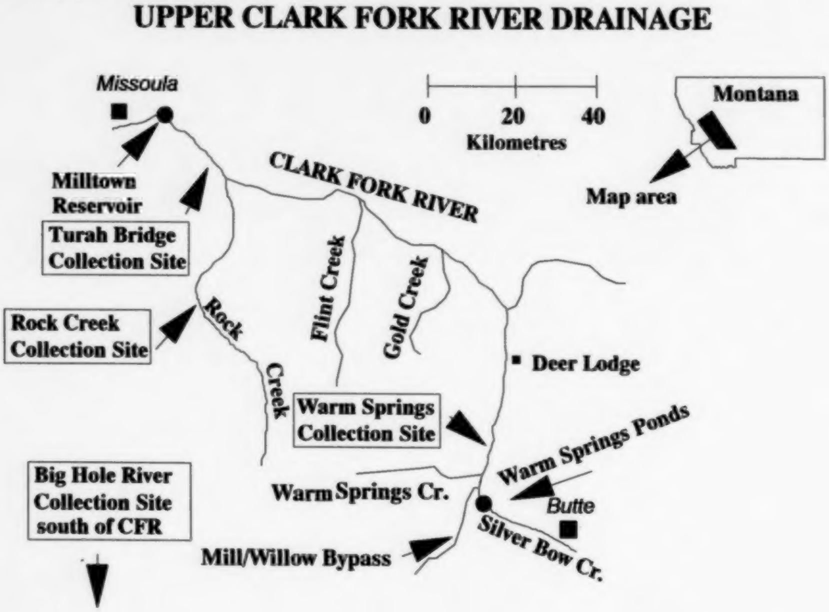
### Necropsy

Length (mm) and weight (g) were recorded for each fish and the condition factor (KTL) was calculated as (weight × $10^5$)/length$^3$. We examined the fish internally and externally for gross abnormalities using the necropsy method described by Goede (1989). For example, opaque eyes, pale skin, eroded fins, and the color and size of organs were noted. This method does not evaluate the cause of abnormalities but is often used to gauge the relative health of hatchery fish.

### Histology

Tissues collected and examined included gill, kidney, liver, spleen, and pyloric caeca. Adipose and pancreatic tissues

**Fig. 1.** Upper Clark Fork River from the confluence of Silver Bow Creek and Warm Springs Creek to Missoula, Montana. Adult brown trout were collected from the Big Hole River and Rock Creek reference sites and from near Turah Bridge and below Warm Springs ponds on the Clark Fork River.



adjacent to pyloric caeca were also examined. All tissues were fixed in Bouin's fixative in the field and transported to the U.S. FWS Fish Technology Center in Bozeman, Montana, where tissues were processed by standard procedures for light microscopy. Sections were cut 4 μm thick, stained with hematoxylin and eosin to examine morphology or with rhodanine to determine the presence of copper (Irons et al. 1977), and examined by light microscopy.

### Lipid peroxidation

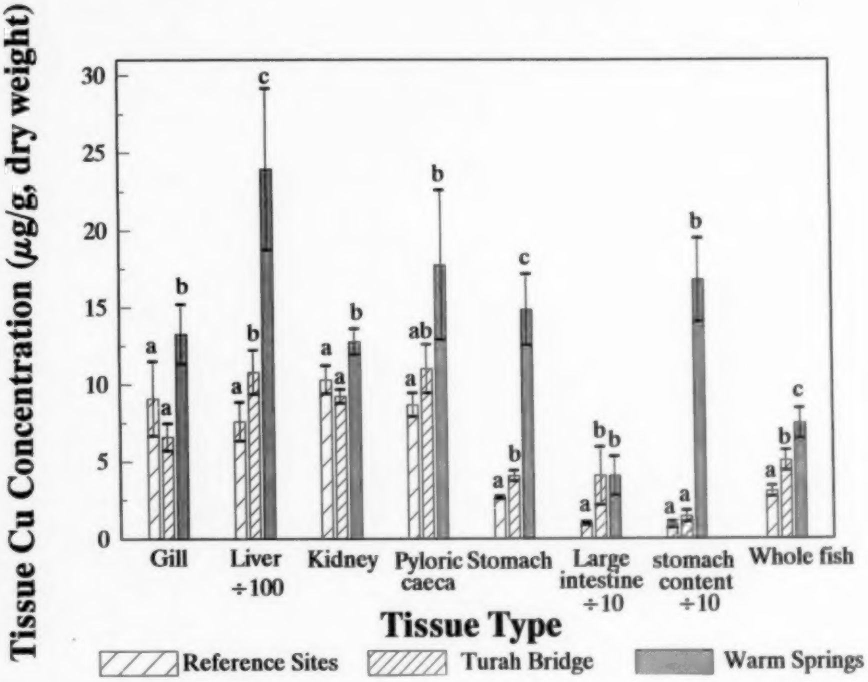
Peroxides are formed in polyunsaturated fatty acids during lipid peroxidation. The peroxides can react with proteins to alter the structure of membranes and form fluorophores. These fluorophores (products of lipid peroxidation) can be measured with a fluorometer. A fluorometric assay (Dillard and Tappel 1984; Fletcher et al. 1973) was used to measure products of lipid peroxidation. A chloroform–methanol extraction of tissue preceded the fluorometric measurement. Two hundred milligrams of ground sample was placed in a glass homogenizer and a 2:1 mixture of HPLC-grade chloroform:methanol was added (7 mL for a 200-mg sample). The tissue was processed four times in the homogenizer with a glass pestle, diluted with an equal volume of water, and homogenized four additional times. The mixture was then vortexed for 2 min and transferred to a Corex tube.

The mixture was centrifuged at 800 × *g* for 1 min and the chloroform layer was removed. Fluorescence was measured (Aminco-Bowman SPF) at a wavelength of 425 nm emission during excitation at 340 and 360 nm. Previous measurements demonstrated that these wavelengths provided the greatest sensitivity and reliability (A.M. Farag, M.A. Stansbury, and H.L. Bergman, unpublished data) and two excitation wavelengths were used to ensure that the measurements were reproducible. The fluorometer transmits the excitation wavelength to the sample, causing it to fluoresce. Fluorescence intensity at the emission wavelength is then measured as relative intensity on the photomultiplier.

### Metallothionein

Metallothionein was measured (in μg/g) in liver and gill samples quick frozen in liquid nitrogen in the field. Five hundred microlitres of Tris buffer (pH 8.0, 1°C) was added to a 500-mg aliquot of ground tissue and centrifuged at 5000 × *g* for 10 min at 4°C. The supernatant was frozen in liquid nitrogen and stored at −70°C for MT analysis by a competitive double-antibody radioimmunoassay (RIA). The RIA developed by Hogstrand and Haux (1990) and later modified by Hogstrand et al. (1994) was used to measure MT in ground fish tissues. The MT assay used rabbit antiserum raised against MT for yellow perch (*Perca fluviatilis*) as the first antibody, $^{125}$I-labelled rainbow trout MT as a tracer, and goat anti-rabbit IgG as a second antibody. A 10 000 × g supernatant prepared from livers of Cd-injected rainbow trout was used as a MT standard. The MT content of the standard was calibrated against a standard curve prepared from rainbow trout MT (Hogstrand et al. 1995). The working range of the RIA was 10–100 ng rainbow trout MT per assay tube, which corresponds to

**Fig. 2.** Copper concentrations (mean ± 1 SEM, µg/g dry weight) in tissues, stomach contents, and whole fish for adult brown trout from the Clark Fork River and reference sites during May 1992. Rock Creek and Big Hole River values were combined (reference sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at $\alpha = 0.05$, on the basis of the Wilcoxon rank sum test that compares median values. For reference sites $N = 19$, for Turah Bridge $N = 9$, and for Warm Springs $N = 10$ for all tissues except $N = 5$ for whole-body measurements. Note the different scaling factors for liver, intestine, and stomach.



0.6–6.0 µg/g liver wet weight. No purified brown trout MT was available, so rainbow trout MT was also used as a standard for measurements of MT from brown trout. Because there may be structural dissimilarities in the antigenic determinants of MT from both species, the reported values of MT from brown trout in the present study have to be considered as relative.

### Blood ions

Whole blood sampled from the fish in the field was thawed and centrifuged at 236 × $g$ for 5 min. The remaining blood was collected and $Ca^{2+}$, $K^{+}$, and $Na^{+}$ concentrations were measured by flame atomic absorption spectrophotometry (Perkin-Elmer model 2830). Replicates, duplicates, and internal standards were used to verify the method.

### Statistics

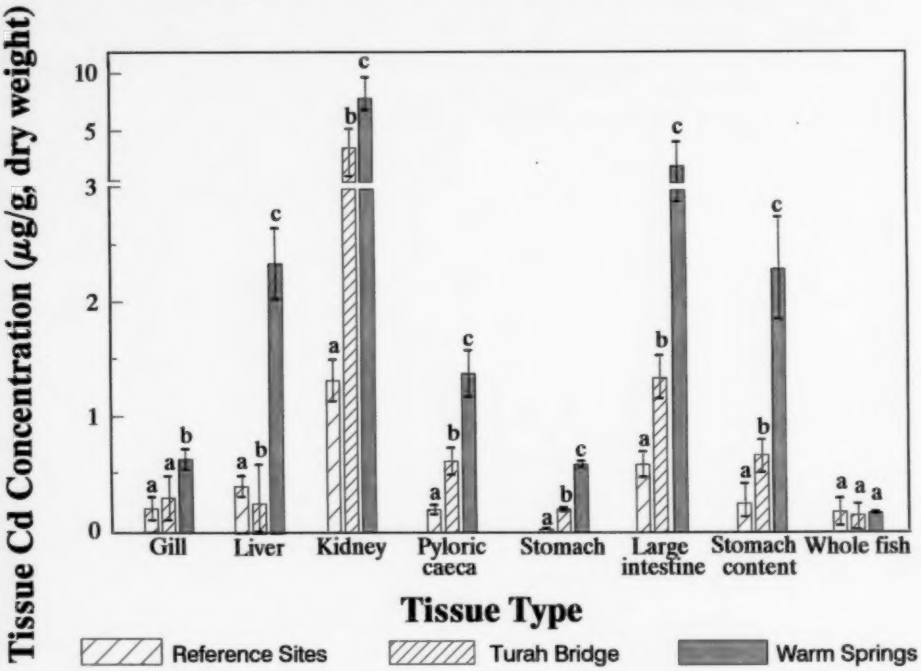
Medians of the tissue metal concentrations were compared with the Wilcoxon rank sum test (Zar 1984) using SAS Institute Inc. (1985) software. Using the TOXSTAT (Gulley et al. 1991) or SAS Institute Inc. (1985) software package, MT, lipid peroxidation, and blood ion measurements were analyzed by a one-way analysis of variance (ANOVA) using sample site as the variable. All ANOVA tests used the Tukey multiple comparison–contrast procedure to compare means. For all tests we used a statistical significance level of 0.05 ($\alpha = 0.05$) and data were transformed (i.e., square, log) when necessary to meet the normality and homogeneity assumptions of an ANOVA. For statistical analyses of data from the field samples, Rock Creek and Big Hole River values were pooled to establish a baseline value (combined reference). To calculate the means, all values less than the detection limit were recorded as the detection limit concentration. However, all of the original data were used to calculate the standard errors of the means (Gilbert 1987; Porter et al. 1988).

## Results

### Metal residues in tissues

We observed significantly elevated concentrations of Cu, As, and Cd in liver, kidney, and pyloric caeca of adult brown trout collected from Warm Springs as compared with the reference sites (Figs. 2–5). In addition, Cu and Cd were significantly elevated in gill, stomach, large intestine, and stomach contents and Cu was also significantly elevated

**Fig. 3.** Cadmium concentrations (mean ± 1 SEM, μg/g dry weight) in tissues, stomach contents, and whole fish for adult brown trout from the Clark Fork River and reference sites during May 1992. Rock Creek and Big Hole River values were combined (reference sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at $\alpha = 0.05$, on the basis of the Wilcoxon rank sum test that compares median values. For reference sites $N = 19$, for Turah Bridge $N = 9$, and for Warm Springs $N = 10$ for all tissues except $N = 5$ for whole-body measurements.



in whole-body samples. In the results presented in Figs. 2–5, mean values + SEM are shown to represent the traditional comparator statistics (means) along with a demonstration of variability (SEMs). However, the results of actual statistical comparisons presented in the figures are from the nonparametric Wilcoxon rank sum test for medians (see Methods section). In these statistical comparisons of median metal residues for individual tissues by site, the comparisons are between reference values (the combined reference that consists of measurements from fish collected from both Rock Creek and Big Hole River) and values from the Clark Fork River sites.

### *Copper*

All tissues measured in fish from Warm Springs had significantly elevated concentrations of Cu when compared with tissues from fish from the reference sites (Fig. 2). Liver, stomach, large intestine, and whole-body samples collected from Turah Bridge also had elevated concentrations of Cu when compared with the same tissues from fish from the reference sites. The greatest differences in Cu concentrations were observed in the stomach tissue and stomach contents that were 4 and 5 times higher, respectively, in fish collected from below Warm Springs ponds than in those in fish from the reference sites. The highest concentrations of tissue Cu were observed in the liver. Livers of fish from below Warm Springs ponds had 2394 μg Cu/g dry weight whereas livers from the reference sites had 759 μg Cu/g dry weight (Fig. 2).

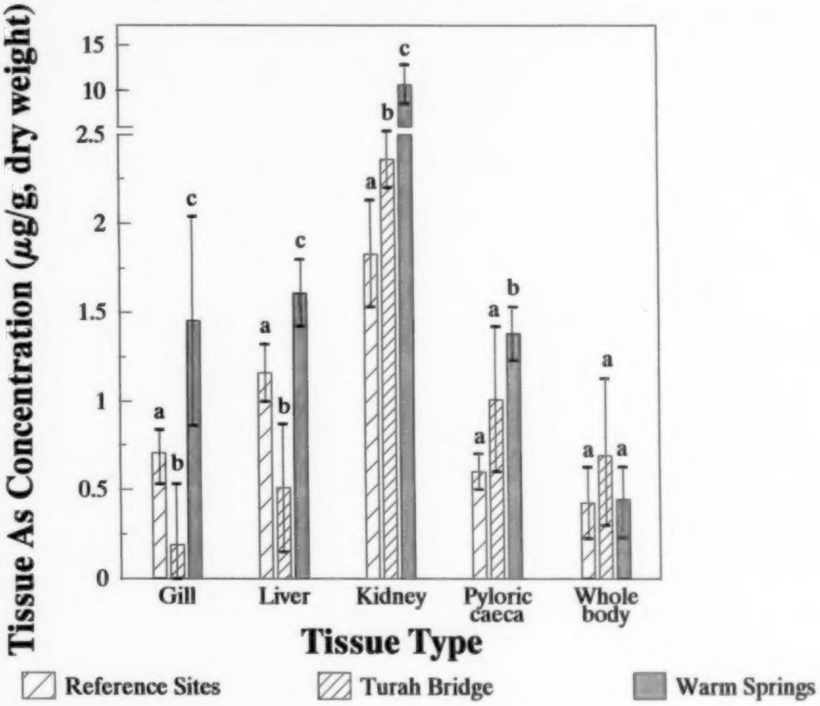
### *Cadmium*

Cadmium concentrations were significantly elevated in gill, liver, kidney, pyloric caeca, stomach, large intestine, and stomach contents of brown trout from below Warm Springs compared with those from the reference sites (Fig. 3). Concentrations of Cd in the kidney, pyloric caeca, stomach, large intestine, and stomach contents of fish from Turah Bridge were also significantly higher than in the same tissues of fish from the combined reference sites. The greatest concentrations of Cd were observed in the kidney; fish from near Turah Bridge and below Warm Springs had mean concentrations of 4.4 and 8.6 μg Cd/g dry weight, respectively, compared with 1.33 μg Cd/g dry weight observed in fish from the combined reference sites. There were no significant differences in whole body Cd concentrations among sites.

### *Arsenic*

Arsenic concentrations in the gill, liver, kidney, and pyloric caeca of brown trout collected from below Warm Springs

**Fig. 4.** Arsenic concentrations (mean ± 1 SEM, μg/g dry weight) in tissues and whole fish for adult brown trout from the Clark Fork River and reference sites during May 1992. Rock Creek and Big Hole River values were combined (reference sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at $\alpha = 0.05$, on the basis of the Wilcoxon rank sum test that compares median values. For reference sites $N = 19$, for Turah Bridge $N = 9$, and for Warm Springs $N = 10$ for all tissues except $N = 5$ for whole-body measurements.



were significantly elevated above those from the reference sites (Fig. 4). The kidney accumulated the highest residues of As; kidneys of fish from below Warm Springs had 11.3 μg As/g dry weight compared with 1.8 μg As/g dry weight for those of fish from the combined reference. Kidneys of fish from Turah Bridge also had significantly higher residues of As than those of fish from the combined reference. The Wilcoxon rank sum test detected a significant difference between pyloric caeca of fish from below Warm Springs and those from near Turah Bridge in spite of the large variability (SEM) around the mean in samples from Turah Bridge (0.4 μg As/g dry weight). Because the Wilcoxon rank sum test compares medians, the concentrations of As in the pyloric caeca of fish collected from below Warm Springs were significantly higher than the concentrations in the pyloric caeca in fish from near Turah Bridge, although the SEMs overlapped. Arsenic levels in the gill and liver of fish from Turah Bridge were significantly lower than in fish from the reference sites. There was no significant difference in the concentrations of As in whole-body tissues. Concentrations of As were not measured in stomach, large intestine, or stomach contents.

*Lead*

The concentrations of Pb in the stomach and stomach contents of fish from below Warm Springs were significantly higher than in the stomachs and stomach contents of fish collected from Turah Bridge and the reference sites (Fig. 5). The concentrations of Pb in the kidneys of fish from near Turah Bridge and below Warm Springs were significantly higher than in the kidneys of fish collected at the reference sites. However, the Pb concentration in pyloric caeca from fish from the reference sites was significantly greater than from fish collected near Turah Bridge. We measured a very high concentration of Pb (39 μg/g dry weight) in one pyloric caeca sample collected from Rock Creek; it is likely that this sample was contaminated with Pb. The Wilcoxon rank sum test did not detect a significant difference in lead levels between pyloric caeca of fish from the reference sites and those of fish from below Warm Springs in spite of a marked difference in the mean values (the median value was not markedly affected by the one extremely high value, while the mean value was affected). Because the Wilcoxon rank sum test compares medians, the concentrations of Pb in the stomachs of fish collected

**Fig. 5.** Lead concentrations (mean ± 1 SEM, µg/g dry weight) in tissues, stomach contents, and whole fish for adult brown trout from the Clark Fork River and reference sites during May 1992. Rock Creek and Big Hole River values were combined (reference sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at α = 0.05, on the basis of the Wilcoxon rank sum test that compares median values. For reference sites *N* = 19, for Turah Bridge *N* = 9, and for Warm Springs *N* = 10 for all tissues except *N* = 5 for whole-body measurements.

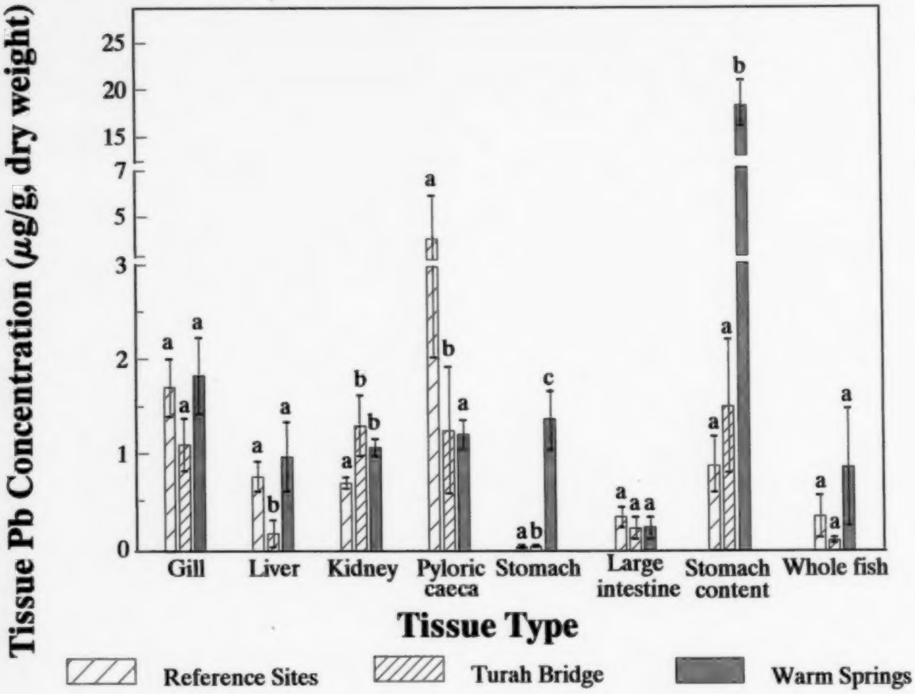


**Table 1.** Weight (g), length (mm), and condition factor (KTL = (weight × $10^5$)/length$^3$) of adult brown trout collected from the Clark Fork River and reference sites during May 1992.

| | *N* | Weight (g) | Length (mm) | KTL |
|---|---|---|---|---|
| Reference sites | | | | |
| Rock Creek | 9 | 647±104 | 397±26 | 0.93±0.64 |
| Big Hole River | 10 | 562±56 | 377±16 | 0.41±0.14 |
| Combined reference | 19 | 603±57*a* | 386±15*a* | 0.69±0.31*a* |
| Clark Fork sites | | | | |
| Turah Bridge | 9 | 644±93*a* | 400±24*a* | 0.46±0.16*a* |
| Warm Springs | 10 | 596±40*a* | 396±9*a* | 0.31±0.04*a* |

**Note**: Values are given as the mean ± 1 SEM. Rock Creek and Big Hole River values were combined (combined reference) for the means comparisons. Means followed by the same letter are not significantly different at α = 0.05.

from Turah Bridge were significantly higher than in the stomachs of fish from the reference sites, although the mean concentrations were the same. There were no significant differences in gill, large intestine, or whole-body Pb concentrations among sites.

### Necropsy assessment

We noted little difference in parameters measured during the necropsy assessment of adult brown trout collected in the field. The conditions of the eye, gill, pseudobranch, hind gut, kidney, liver, fin, and opercula were similar in fish collected from the four sites. The most notable difference found during the necropsy assessment was that the KTL was reduced in brown trout collected from below Warm Springs (0.31) compared with the combined reference sites (0.69), but this was not significant (Table 1). There were no significant differences in the weights or lengths of fish among sites.

### Histology

Most of the cellular changes observed in the four groups of fish are commonly seen in older fish. Numerous splenic hematomas, degeneration of individual hepatocytes, abundant melanomacrophages, and sclerosis of renal glomeruli were noted in all groups of fish examined. However, groups of fish were distinguishable on the basis of pathological

**Table 2.** Lipid peroxidation of tissues sampled from adult brown trout from the Clark Fork River and reference sites during May 1992.

| | Gill | | Kidney | | Large intestine | | Liver | | Pyloric caeca | | Stomach | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Relative intensity | *N* | Relative intensity | *N* | Relative intensity | *N* | Relative intensity | *N* | Relative intensity | *N* | Relative intensity |
| Reference sites | | | | | | | | | | | | |
| Rock Creek | 8 | 0.16±0.01 | 7 | 0.66±0.05 | 8 | 0.12±0.03 | 8 | 0.05±0.02 | 8 | 0.02±0.01 | 9 | 0.20±0.02 |
| Big Hole River | 9 | 0.18±0.02 | 8 | 0.92±0.15 | 10 | 0.25±0.03 | 10 | 0.06±0.02 | 8 | 0.07±0.02 | 10 | 0.23±0.03 |
| Combined reference | 17 | 0.17±0.01*a* | 15 | 0.80±0.09*a* | 18 | 0.19±0.03*a* | 18 | 0.06±0.01*a* | 16 | 0.05±0.01*a* | 19 | 0.22±0.02*a* |
| Clark Fork sites | | | | | | | | | | | | |
| Turah Bridge | 8 | 0.23±0.03*a* | 7 | 0.86±0.07*a* | 9 | 0.27±0.06*a* | 8 | 0.08±0.02*a* | 9 | 0.07±0.02*a* | 9 | 0.21±0.04*a* |
| Warm Springs | 9 | 0.20±0.03*a* | 9 | 0.77±0.04*a* | 10 | 0.36±0.02*b* | 10 | 0.27±0.03*b* | 10 | 0.21±0.02*b* | 10 | 0.23±0.03*a* |

**Note**: Values are given as the mean ± SEM. Lipid peroxidation is expressed as the fluorometric measurement (relative intensity) of a chloroform extract of tissue. The relative intensity was measured at 360 nm excitation and 425 nm emission when 1.0 μg/mL of quinine sulfate measured 12.5. Rock Creek and Big Hole River values were combined (combined reference) for the means comparisons. Means followed by the same letter (within a tissue) are not significantly different at α = 0.05.

**Table 3.** Metallothionein concentrations (μg/g wet weight) in liver and gill samples of adult brown trout from the Clark Fork River and reference sites during May 1992.

| | Liver | | Gill | |
|---|---|---|---|---|
| | *N* | [Metallothionein] | *N* | [Metallothionein] |
| Reference sites | | | | |
| Rock Creek | 7 | 168.0±18.4 | 7 | 26.1±1.6 |
| Big Hole River | 9 | 191.7±39.0 | 9 | 23.3±2.1 |
| Combined reference | 16 | 181.3±22.9*a* | 16 | 24.3±1.3*a* |
| Clark Fork sites | | | | |
| Turah Bridge | 7 | 132.8±15.0*a* | 7 | 24.0±1.3*a* |
| Warm Springs | 9 | 422.0±128.7*b* | 9 | 22.1±1.8*a* |

**Note**: Values are given as the mean ± SEM. Rock Creek and Big Hole River values were combined (combined reference) for the means comparisons. Means followed by the same letter (within a tissue) are not significantly different at α = 0.05.

differences as noted below. Livers of fish collected from the Warm Springs, Turah Bridge, and Rock Creek sites (but not the Big Hole River site) contained variable amounts of Cu inclusions within the cytoplasm of hepatocytes.

Liver sections from one of eight fish collected from Rock Creek were lightly positive for Cu (few focal areas within hepatocytes containing small granules that stained positive for Cu). Liver sections of two of nine fish from Turah Bridge stained positive for Cu: one stained mildly, and one moderately. Liver sections from 8 of the 10 fish collected from Warm Springs contained intracytoplasmic granules that stained positive for Cu: 4 stained mildly, 1 moderately, and 3 extensively. In addition, nuclear vacuolation of hepatocytes was noted in three fish collected from Rock Creek, seven fish collected from Turah Bridge, and four fish collected from Warm Springs.

### Lipid peroxidation

Differences in lipid peroxidation were observed among the brown trout from the different field sites for large intestine, liver, and pyloric caeca (Table 2). Lipid peroxidation was significantly greater in the large intestine, liver, and pyloric caeca of fish collected from below Warm Springs than in the same tissues of fish from Turah Bridge and the reference sites.

### Metallothionein

Livers of fish from below Warm Springs had a significantly greater concentration of MT than the livers of fish from Turah Bridge or the reference sites (Table 3). The concentration of MT in the livers of fish from below Warm Springs was more than twice the concentration measured in fish from the reference sites. There were no significant differences in the concentrations of MT in gills (Table 3).

### Blood ions

The concentration of $Na^+$ in the blood was significantly lower in fish from Turah Bridge than in fish from the combined reference sites and in fish from below Warm Springs (Table 4). The concentration of blood $Ca^{2+}$ was significantly

**Table 4.** Blood ion concentrations of adult brown trout from the Clark Fork River and reference sites during May 1992.

| | | Blood ion concentrations (μequiv./L) | | |
|---|---|---|---|---|
| | *N* | Ca$^{2+}$ | K$^{+}$ | Na$^{+}$ |
| Reference sites | | | | |
| Rock Creek | 8 | 3.3±0.1 | 37.7±1.0 | 114.0±1.5 |
| Big Hole River | 10 | 3.2±0.1 | 35.9±1.5 | 107.1±1.7 |
| Combined reference | 18 | 3.3±0.1*a* | 36.7±0.9*a* | 110.5±1.4*a* |
| Clark Fork sites | | | | |
| Turah Bridge | 9 | 3.7±0.2*a* | 38.2±2.2*a* | 98.3±3.4*b* |
| Warm Springs | 10 | 3.0±0.1*b* | 38.9±1.2*a* | 108.0±2.0*a* |

**Note**: Values are given as the mean ± SEM. Rock Creek and Big Hole River values were combined (combined reference). Means followed by the same letter (within an ion) are not significantly different at $\alpha = 0.05$.

lower in fish from Warm Springs than in fish from Turah Bridge or the combined reference sites. There were no significant differences in blood K$^{+}$ levels from fish among all sites.

## Discussion

### Metal residue accumulation

Residues of Cu, As, Cd, and Pb were significantly higher in tissues of adult brown trout collected in May 1992 from the Clark Fork River than in those of adult brown trout from reference sites in Rock Creek and the Big Hole River. Copper was the most highly elevated of the four metals analyzed, and of the metals analyzed, Cu was the only metal that was significantly elevated in whole-body residues. Whole-body Cu residues reported here for Clark Fork River brown trout (Fig. 2) were lower than those measured as early as 1974 for fish collected from the same reaches of the river. For instance, the Anaconda Company collected brown trout and whitefish from below Warm Springs and from several sites further downstream from Warm Springs in 1974 (Dent 1974). The tissues were analyzed by atomic absorption spectroscopy and the company reported whole-body Cu concentrations (μg/g dry weight) of 7.2 below Warm Springs, 7.2 near Racetrack (located ca. 10 river miles downstream from Warm Springs), 7.0 near Rock Creek, and 4.5 below Drummond (located ca. 6.6 river miles downstream from Warm Springs). Results of more recent analyses of Cu residues in Clark Fork River brown trout tissues are very similar to the liver, gill, and kidney residue concentrations reported here for May 1992 (Fig. 2 and Table 5). Results from April 1989 (Phillips and Spoon 1990), August 1991, and November 1991 (G. Phillips, Montana Department of Fish, Wildlife and Parks, Helena, Mont., personal communication) for brown trout from below Warm Springs include mean concentrations (μg/g dry weight) of 1635–2049 for liver, 2.9–16.6 for gill, and 8.5–11.4 for kidney.

The copper residue concentrations reported for fish from various sites in the United States are substantially lower than the concentrations observed in fish from the Clark Fork River. As summarized in Table 5, Schmitt and Brumbaugh (1990) reported a national geometric mean of 3.25 μg/g and a national 85th percentile concentration of 5.0 μg/g for the Cu concentration in whole fish from 109 U.S. sites (concentrations from Schmitt and Brumbaugh's wet weight residue values were multiplied by 5 to estimate dry weight residue values; assumes 80% moisture, William Brumbaugh, National Biological Service, Columbia, Mo., personal communication, and unpublished data from this study). Both Turah Bridge and Warm Springs fish samples were above the national geometric mean and Warm Springs fish samples were above the 85th percentile concentrations (Table 5).

These highly elevated residues in Clark Fork River fish have been present for at least the past 20 years and undoubtedly since fish began to recolonize the upper Clark Fork River during the 1960s. The concentrations of copper in the livers of fish collected from the reference sites were higher than expected. It is possible that fish from the upper Clark Fork River migrated into Rock Creek and less-established mining activities may have taken place along the Big Hole River. Because of high tissue metals in fish from the reference sites, our findings are based on fish collected from conservative reference sites.

### Physiological malfunctions and structural lesions

In conjunction with the significantly elevated metal residue concentrations described above for adult brown trout collected from the Clark Fork River, we evaluated physiological and structural (histopathological) changes in tissues from these same fish samples. We observed lower concentrations of Na$^{+}$ in the blood of fish from Turah Bridge and lower concentrations of Ca$^{2+}$ in the blood of fish from below Warm Springs than in fish from the combined reference sites. However, the most marked abnormalities were observed in the concentrations of lipid peroxidation products and MT and in the tissue lesions.

The high concentrations of lipid peroxidation products we measured in the fish from below Warm Springs ponds indicate that cell death and possibly tissue damage occurred in the liver, pyloric caeca, and large intestine of these fish. The polyunsaturated fatty-acid side chains present in cell membranes are the targets of lipid peroxidation (Halliwell and Gutteridge 1985). If these side chains are peroxidized, the integrity of the cell membrane is diminished and the balance of fluidity and structure in the cell membrane is compromised. Such changes in the structural integrity of cell membranes may ultimately result in tissue damage and cell death (Halliwell and Gutteridge 1985; Wills 1985).

Metals such as Cu that exist in more than one valence state can be catalysts for lipid peroxidation (Wills 1985). Stern (1985) reported that copper, sulfhydryl groups, and oxygen seem to interact in biological systems to initiate lipid peroxidation. Metals can also cause lipid peroxidation because they inhibit important antioxidant enzymes such as glutathione peroxidase and transferase (Splittgerber and Tappel 1979; Reddy et al. 1981; DiGiulio et al. 1989).

Lipid peroxidation has been documented as a result of both water and dietary metal exposures. Radi and Matkovics (1988) measured increased lipid peroxidation in liver, gill, and muscle of carp exposed to $CuSO_4$ in the water and Sokol et al. (1990) measured increased lipid peroxidation

**Table 5.** Comparison of tissue residue concentrations of Cu in fish from the Clark Fork River and reference sites with representative background samples and laboratory effect studies.

| | Tissue residues (μg/g dry weight) | | | | | |
|---|---|---|---|---|---|---|
| | Whole fish | Liver | Gill | Kidney | Comments | Reference |
| This study[a] | | | | | | |
| Reference | 3.0 | 758.9 | 6.6 | 10.3 | Brown trout adults | This study[a] |
| Turah Bridge | 4.3[b] | 1078.8[b] | 6.6 | 9.2 | Brown trout adults | This study[a] |
| Warm Springs | 6.4[b] | 2393.6[b] | 13.3[b] | 12.8[b] | Brown trout adults | This study[a] |
| Background samples | | | | | | |
| National geometric mean | 3.3[c] | — | — | — | 315 samples, 109 sites, mixed species | Schmitt and Brumbaugh 1990 |
| National 85th percentile | 5.0[c] | — | — | — | 315 samples, 109 sites, mixed species | Schmitt and Brumbaugh 1990 |
| Lake Superior | — | 28 | — | — | Lake trout | Lucas et al. 1970 |
| Lake Michigan | — | 21 | — | — | Lake trout | Lucas et al. 1970 |
| Lake Huron | — | 80[c] | — | — | Several species | Brown and Chow 1977 |
| Laboratory effect studies | | | | | | |
| 88-d water–food exp. | | | | | | |
| Brown trout alevins | 6.4–44.7 | 147–268 | — | — | Range of concentrations with significant effects on growth | Woodward et al. 1995 |
| Rainbow trout alevins | 15.9–22.5 | 88–125 | — | — | Range of concentrations with significant effects on growth | Woodward et al. 1995 |
| 22-month water exp. | | | | | | |
| Bluegill adults (162 μg/L) | — | 480 | 13 | 44 | Significant effects on survival growth, or reproduction | Benoit 1975 |
| Bluegill adults (77 μg/L) | — | 57 | 6 | 12 | No significant effects on survival, growth, or reproduction | Benoit 1975 |
| 24-month water exp. | | | | | | |
| Brook trout adults (9.4 μg/L) | — | 238 | 6.7 | 16.5 | No significant effects on survival, growth, or reproduction | McKim and Benoit 1974 |

[a] Numerical values contained in Bergman (1993).

[b] Significantly greater than value at reference site ($p \leq 0.05$).

[c] Copper concentrations reported on a wet weight basis multiplied by 5 to estimate dry weight concentrations (assumes fish are 80% water, William Brumbaugh, National Biological Service, Columbia, Mo., and unpublished data from this study).

in the hepatic mitochondria of rats following a dietary exposure of 1000 μg Cu/g for 8 weeks. Woodward et al. (1995) also observed significantly increased concentrations of products from lipid peroxidation in early life-stage brown trout fed an invertebrate diet collected from below Warm Springs ponds, and Farag et al. (1994) observed elevated lipid peroxidation products in the kidneys of adult rainbow trout (*Oncorhynchus mykiss*) fed for 3 weeks on a diet collected from below Warm Springs ponds. Our observations of high concentrations of products of lipid peroxidation in tissues of fish collected from below Warm Springs ponds substantiate the theory that lipid peroxidation is one mechanism by which metals may cause toxicity. The theory of a lipid peroxidation toxicity mechanism is supported further by the trend of higher concentrations (though not significantly higher) of products of lipid peroxidation in tissues from fish collected from Turah Bridge than in fish collected from the reference sites.

MTs are metal-binding proteins that bind metals, such as Cu, Cd, and Zn (Stegeman et al. 1992). MT concentrations in livers, but not gills, of brown trout collected from below Warm Springs were twice as high as those in brown trout from the combined reference sites. Woodward et al. (1995) did not observe a significant increase in MT concentrations as a result of metal exposure in their experiments; however, the small size of those fish necessitated analysis of whole fish. Because MT is predominantly present in the liver and kidney tissues (Olsson and Hogstrand 1987), any differences may have been masked in the Woodward et al. (1995) whole-body determinations. Increased MT concentrations in fish liver and gill following controlled exposures to metals have been well documented (Reichert et al. 1979; Dixon and Sprague 1981*a*; Spry and Wood 1989) and these increases have been associated with decreased growth (Dixon and Sprague 1981*a*, 1981*b*; Roch and McCarter 1984). Roch et al. (1982) also documented high MT concentrations in fish from the Campbell River watershed in British Columbia, which was contaminated with metals. Deniseger et al. (1990) noted a parallel decline of both MT concentrations in the livers of fish and metals concentrations in the water. The authors suggested that the biological recovery lagged behind the improvements in water quality and suggested that MT be monitored as an indicator of fish health. The high concentrations of MT measured in fish livers during this study may also decrease as concentrations of metals in the surface waters and sediments of the Clark Fork River decline.

A series of lesions were observed during the histopathologic examination of fish tissues collected from the field. The main microscopic lesions in tissues of adult brown trout from the Clark Fork River sites were Cu inclusions in hepatocyte cytoplasm and vacuolation of hepatocyte nuclei. The same but less severe lesions were observed in several fish from Rock Creek; however, none of the fish from the Big Hole River exhibited these pathologies. The localization of Cu in hepatocyte cytoplasm from these Clark Fork River brown trout emphasizes the importance of the extremely high liver Cu residues reported above for these same fish, because actual deposition of Cu intracellularly in the liver explains diversion of cell metabolism to MT synthesis (significantly elevated liver MT concentrations described above), and implies severe disruption of cell function. In fact, nuclear vacuolation observed in hepatocytes from many of these same fish is a sign of irreversible cell destruction and eventual cell death most likely by karyolysis (Charles Smith, U.S. FWS Fish Technology Center, Bozeman, Mont., personal communication). Calventi and Nigrilli (1961) also observed vacuolation of hepatocytes in fish following exposure to waterborne Cu.

### Growth

We did not observe significant differences in size but we did not collect scales to determine the specific age-classes of fish and it may be necessary to have a sample size greater than 10 to observe size differences in the field. Tohtz (1992) collected brown trout from the Clark Fork and Big Hole rivers and calculated lengths at annulus formation. In two age-classes (1982 and 1983), mean length at annulus formation was significantly larger in Big Hole River fish than in fish from the Clark Fork River. Woodward et al. (1994, 1995) observed significant reductions in the growth of early life-stage brown trout and rainbow trout when the fish were exposed to dietary metals from the Clark Fork River. By the end of these studies, the magnitude of these differences was so great that it could be observed by a simple visual inspection of the fish.

A cause–effect linkage between metal exposures typical of the Clark Fork River and injury to fish is further substantiated if we consider the other information collected in this study, data gathered during other studies on the Clark Fork River, and available relevant information from the literature. For instance, for fish exposed to Clark Fork River metals in the laboratory or field, we have presented results showing elevated concentrations of lipid peroxidation products (this study; Woodward et al. 1995; Farag et al. 1994), elevated concentrations of tissue MT (this study), increased scale loss (Farag et al. 1994), and significantly elevated tissue metal residues (this study, Woodward et al. 1994, 1995; Farag et al. 1994). A metabolic cost to fish can be associated with all of these measurements, but the most complete links available in the published literature between metal exposure and effects on growth relate to MT concentrations and tissue metal residues.

Laboratory studies (Dixon and Sprague 1981*a*, 1981*b*) have linked Cu exposure, elevated MT, acclimation to Cu, and reduced growth in rainbow trout and brown trout. In all waterborne Cu exposures in which MT was significantly elevated, the investigators also observed a significant reduction in growth. Dixon and Sprague (1981*b*) concluded that the reduced growth that they observed represented the increased metabolic costs associated with acclimation to Cu. Together, the physiological responses measured in brown trout from below Warm Springs provide a plausible explanation for the metabolic costs of metal toxicity. Significantly elevated liver MT levels, significantly elevated lipid peroxidation in several tissues, marked nuclear vacuolation in hepatocytes, intracellular Cu granule deposition in hepatocytes, and significantly elevated liver and whole-body Cu residue accumulation likely require energy that could otherwise be used for normal growth and development of brown trout in the Clark Fork River.

In a series of long-term studies in which fish were exposed to waterborne Cu, elevated liver Cu residues were always associated with significant effects on growth, survival, or reproduction (Table 5). On the basis of these studies, liver Cu concentrations between 238 and 480 μg Cu/g seem to be detrimental to growth and reproduction. Although variables associated with water and dietary exposure to Cu make it difficult to establish precise thresholds for effects, the liver Cu concentrations measured in brown trout collected from below Warm Springs far exceed those at which effects on growth and reproduction have been demonstrated in the laboratory. Moreover, the concentrations of Cu in livers of fish from the reference sites are also above this laboratory-derived threshold, and growth and reproduction of these fish may also be affected.

Concentrations of other metals were also elevated in brown trout collected from the Clark Fork River from below Warm Springs. We observed significantly higher tissue concentrations of As, Cd, and Pb in brown trout collected from the Clark Fork River than in fish collected from the reference sites. These additional metals may further compromise the health of fish in the Clark Fork River as researchers have linked increased tissue levels of As (Cockell and Hilton 1988), Cd (Benoit et al. 1976), and Pb (Holcombe et al. 1976) with decreased survival and growth of rainbow trout and brook trout.

The high concentrations of Cu and other metals in tissues of brown trout collected from the Clark Fork River indicate that the health of these fish is impaired. Metal residues measured in tissues collected from brown trout in this study have been associated with effects on growth and reproduction in published laboratory studies. In particular, the liver concentrations of Cu in fish collected from the Clark Fork River exceed a threshold at which effects on growth and reproduction have been observed by other researchers. In addition to the significantly elevated tissue metal residues, we have also measured other physiological malfunctions in the field and the laboratory, including elevated lipid peroxidation and metallothionein concentrations (this study) and histological changes (this study; Woodward et al. 1995), which support the conclusion that fish health in the Clark Fork River is impaired. Furthermore, the high concentrations of metals in the Clark Fork River may be typical of rivers in the western United States where discharges from mining activities have occurred since the late 1800s. While the metals of concern may vary in rivers throughout the west, results from this study can be used as a guideline for future research.

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PERSPECTIVE

# Fisheries Management Science: a plea for conceptual change

**Robert L. Stephenson and Daniel E. Lane**

**Abstract**: Recent fishery failures, combined with changing views on management, point to the critical and urgent need for a new approach to fisheries management. Future management should focus on integrated fisheries, rather than solely on fish populations, and will require an appropriate combination of biological considerations with operational, social, and economic considerations of the fishery. This requires development of both a conceptual framework and an appropriate methodology for interdisciplinary decision making in fisheries management. We propose integration of the traditional fields of fisheries science and management with the scientific approach of management science to form Fisheries Management Science. Fisheries management science provides the framework and methodologies for defining multiple objectives and constraints, modelling alternative management scenarios, and assessing and managing risk. This framework accepts diverse information sources toward anticipatory decision making and consensus building, and offers a new paradigm within which effective fisheries management can emerge.

**Résumé** : Les échecs récents des pêches et l'évolution des points de vue en matière de gestion des pêches soulignent l'importance et l'urgence d'une nouvelle approche. La nouvelle gestion devra mettre l'accent sur l'intégration des pêches, et non seulement sur les populations de poisson, et devra permettre de combiner adéquatement les facteurs biologiques aux facteurs opérationnels, sociaux et économiques des pêches. Cela suppose l'élaboration conjointe d'un cadre conceptuel et d'une méthodologie pour la prise de décisions interdisciplinaires en gestion des pêches. On propose une intégration des sciences halieutiques et de la gestion des pêches avec le champ des sciences de la gestion le tout dans le but d'obtenir la Science de la Gestion des Pêches. La science de la gestion des pêches fournit un cadre et des acquis méthodologiques pour définir des objectifs et des contraintes multiples, pour modéliser divers scénarios, et pour évaluer les risques de gestion. Ce cadre autorise des sources d'information diverses pour la prise de décisions anticipatoires et l'obtention de consensus tout en offrant un nouveau paradigme d'où il serait possible de dégager une gestion efficace des pêches.

## Introduction

The past four decades have seen considerable innovation and development in the management and regulation of fisheries worldwide. However, this modern fisheries management experiment has been only partially successful. Recent fishery failures, combined with changing views on management philosophy, point to the critical and urgent need for a new approach. Shortcomings of current fisheries management systems include the inability to make analytical decisions that account for multiple objectives and the inherent variability of fisheries systems, and that incorporate socioeconomic considerations. An emphasis on socioeconomics integrated with ecology will require a modified methodology to evaluate management options against diverse management objectives including conservation, economic, social, and political considerations. This methodology provides a framework for structured decision making employing the techniques of operations research and management science.

This paper provides a critique of the current state of fisheries science and management, and provides a perspective for the required new approach that we term Fisheries Management Science. We propose that fisheries management science is an appropriate context in which to develop strategic alternatives and to evaluate these in terms of biological, economic, social, and operational management objectives.

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## Failure of the modern fisheries management experiment

Fisheries management has evolved over the past four decades as an entity in its own right (Royce 1989; Parsons 1993). Prior to World War II there was almost exclusive emphasis on fisheries development and modernization. In

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the years since World War II, a period of high exploitation and continued improvement in fishing technology, there has been an emphasis on increasing regulation to restrict fishing. Attempts to adapt management to rapid industrial change constitute the modern fisheries management experiment. In Canada, for example, the 1960s saw a change from a strategy of developing commercial fisheries to a strategy of managing the twin problems of overfishing (conservation) and overcapacity (economic viability) (Parsons 1993, p. 2). During this period, fisheries management worldwide has come to be identified with a variety of restrictive measures such as limited entry, catch restrictions (e.g., total allowable catches or TACs), extended territorial jurisdiction, and property rights (see for example Stephenson et al. 1993; Parsons 1993).

In spite of these measures and considerable public spending annually in the regulation and research of fisheries, there have been many management failures. Catastrophic collapses of fish stocks, with serious social impacts, have continued to occur. Examples of failures in recent decades include the collapse of California sardine (Radovich 1981) and the spectacular crash of Peruvian anchoveta stocks (Hilborn and Walters 1992) in the Pacific, and the demise of North Sea (Burd 1991) and Georges Bank (Anthony and Waring 1980) herring stocks in the Atlantic. A vivid current example is the continued closure of major groundfish fisheries in Atlantic Canada as a result of historically low stock levels (Fisheries Resource Conservation Council 1993; Parsons 1993).

This modern fisheries management experiment is nearly a half century old (Larkin 1988; Royce 1989) and a number of conferences and papers have pointed out shortcomings (e.g., Sutinen and Hanson 1986; Wooster 1988; Miles 1989). There continue to be widespread signs of dissatisfaction with current fisheries management systems throughout the world; for example, institutional reorganization and reform in Canada (Parsons 1993), debate and change within the International Council for the Exploration of the Sea (ICES) regarding the form and format of advice (Serchuk and Grainger 1992), problems in the United States with implementation of management plan guidelines as part of the Magnuson Fisheries Conservation Management Act (Marasco and Miller 1988), and increasing prevalence of revolution style management situations in fisheries (Gale 1992).

The limitations of current approaches to fisheries management are complex, but common characteristics include the following: (*i*) an inability of management regimes to deal with the inherent variability of the environmental, biological, and economic aspects of fisheries systems (Larkin 1978; Magnuson 1991); (*ii*) failure to define longer term management goals that meet specific biological, social and economic objectives and targets for fisheries (Serchuk and Smolowitz 1990); (*iii*) a lack of year-over-year accountability in management decision making and an inability to react and anticipate change, for example, in industry adjustment to regulations and changing market conditions (Lane 1992*a*); (*iv*) predominance of biological advice that does not adequately address stochastic impacts and lacks appropriate economic, social, or operational considerations (Larkin 1988); (*v*) lack of effective involvement by stakeholders and vested interest groups in fisheries management decision making (Pearse and Walters 1992); and (*vi*) pressure by government regulatory agencies to rationalize and improve efficiency as cost-cutting mechanisms (Parsons 1993).

## The elements of improved fisheries management

Improvements to fisheries management have been suggested in the literature and in special symposia over the past two decades (see, for example, Rothschild 1973; Sutinen and Hanson 1986; Wooster 1988; Miles 1989). In general these require overcoming problems associated with (*i*) biological assessments, (*ii*) development and evaluation of management strategies, (*iii*) institutional structures, and (*iv*) the lack of an integrated problem-solving methodology.

### Biological assessments

Biological evaluation of stock status has been the cornerstone of fisheries management. In most situations, management decisions are currently based on scientific advice that is predominantly biological and that is developed by specialized committees (Hilborn et al. 1993). The obvious importance of fisheries biology to conservation, the advanced state of methodology and organization of advisory bodies, the precedence of biological information in the decision-making process, and the relative lack of contribution from other disciplines have allowed biology to dominate fisheries management. Biological advice is often the sole basis for management in the absence of information and comparable analyses from other domains, notably fisheries economics and social considerations. The media and the public have come to equate scientific advice with fisheries management decisions. However, biological evaluation and stock assessment methods are not without problems. Although analytical methods are at an advanced state, the biologists giving advice are only beginning to consider the variability of estimates of stock status and the impact errors in estimates. This problem is confounded by ineffective communication of uncertainty and errors in data (Schnute and Hilborn 1993).

### Management strategies

The post World War II era saw a change in philosophy, from the pursuit of maximum sustainable yield to the pursuit of the more diffuse concepts of best use or optimal yield that were to incorporate biological, economic, and social objectives (Rothschild 1973; Sissenwine 1978; Parsons 1993). Unfortunately, there has not been a mechanism in place to define and evaluate best use. Fisheries management has been more preoccupied with tactics than with strategic planning (Rothschild 1973). Objectives have been broad, ill-defined, and in many cases not operationally feasible. Strategies, where they have existed, have been cast aside or ignored in times of crisis or dispute (e.g., in Atlantic Canada, the departure from the $F_{0.1}$ fishing mortality standard for groundfish stocks to a 50% approach rule arguably "minimized the impact on fishermen of drastic reductions in TACs between consecutive years," Rivard and Maguire 1993). There has been an inability to implement broadly stated social and economic objectives through specific operational goals and targets. Standardized biological reference points have been confused with management objectives, and there have been problems distinguishing between objectives and strategies (e.g., Wooster 1988; Smith et al. 1993). In addition, there has been a lack of fishery-specific targets; standardized fishing mortality policies have been used instead (such as the $F_{0.1}$ reference catch level used in the management of many North Atlantic fisheries).

### Institutional structure

Traditionally, scientific resource evaluation, or stock assessment, has been separate from the operational and political process of management decision making (Hilborn et al. 1993). It has also been advocated that "conservation decisions should be conducted separately and prior to allocation decisions to the greatest possible extent" (Fisheries Resource Conservation Council 1993, p. 21). This suggestion avoids political influence on scientific evaluation of stock status, but leaves a rift between the assessment and management functions. Although recent definitions of management have called for transdisciplinary decision making through direct linkage of fisheries science and fisheries management (Royce 1984; Anderson 1987; Wooster 1988; Pearse and Walters 1992; Hilborn et al. 1993; Stern 1993), this has not yet occurred in practice. Existing institutional arrangements continue to support the separation of fields (O'Boyle 1993).

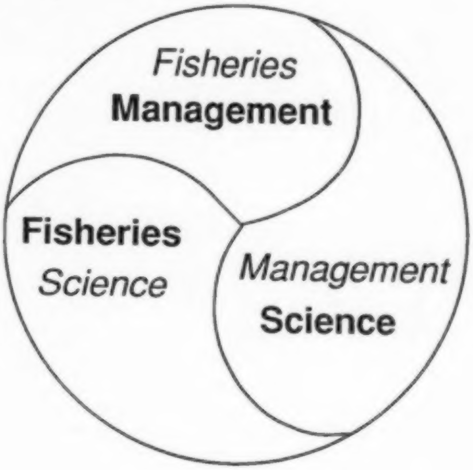
### Methodological framework

Current management approaches have been unsuccessful in addressing and incorporating the range of criteria required to manage multiple objectives. It has been implicitly assumed that social and economic considerations would be added after the independent scientific (or more strictly biological) advice was presented. Socioeconomic considerations, long recognized as important factors in fisheries management, have usually been left to the political decision makers and in this context they often appear to be in opposition to biological advice. There has been no integrated decision-making framework to allow socioeconomic or operational aspects to be reviewed and analyzed in balance with the biological aspects.

The lack of appropriate involvement of industry and the public in fisheries decision making is seen as a critical factor in the failure of modern fisheries management (Pearse and Walters 1992; Hilborn et al. 1993). The fishing industry and the public increasingly want greater input into management of resources. At the same time, governments face prohibitive costs and pressure to improve the efficiency of management. More cooperative management (comanagement and industry–government partnerships) and cost-recovery programs for the regulatory activities of central agencies (for example, the Australian blueprint for management and cost recovery (Commonwealth of Australia 1992)) are inevitable.

Improved fisheries management requires a major conceptual shift. There is, increasingly, the need to integrate and evaluate and to decide among conflicting expectations and demands. There needs to be a mechanism that ensures the management of fisheries, not just fish. There is, also, the need to incorporate stakeholders in a process of comanagement. What is proposed here in response to these needs is the merging of fisheries science, fisheries management, and management science (Fig. 1) toward development of fisheries management science, complete with a specific scientific context and methodology. This requires real conceptual change: a paradigm shift.

**Fig. 1.** The three-part helix connected at the core and linking the three disciplines of fisheries science, fisheries management, and management science symbolizes the integrated, multidisciplinary field of fisheries management science.



## The concept of fisheries management science

The term fisheries science (the application of science, including biological science to fisheries problems; Wooster and Miller 1988) has been almost synonymous with the term fisheries biology (Magnuson 1991) and has been characterized by the disciplines of fish biology (and ecology) and population ecology (stock assessment, resource evaluation). These disciplines have established a large literature and a strong scientific methodology. Employment of professionals in this field (mainly in the public service) is in the tens of thousands, and in North America has approximately doubled in each decade from 1940 to 1980; the field is supported by several major international organizations (including the International Council for the Exploration of the Sea, the North Atlantic Fisheries Organization, and the American Fisheries Society), and more than 120 journals (Royce 1984).

Fisheries management, although often lumped with fisheries science, has a different focus. Fisheries management is the action of designing, justifying, and administering control of fisheries systems (Hilborn and Walters 1992; Parsons 1993). It deals with the interaction between the resource and its use (e.g., "fluctuations in abundance of fishery resources, the role of man in producing such fluctuations, and measures that can be taken to achieve and maintain optimum yields from these resources" (Magnuson 1991)), and should be "anthropocentric" rather than "ichthyocentric" (Larkin 1988). Fisheries management, in contrast to fisheries science, has not developed a standardized scientific methodology. Past management has not formally considered the full suite of implications that its policies engender, and as a result it has failed to live up to its broad mandate.

As a separately evolving discipline, fisheries management must develop a methodology to integrate biological considerations with the economics of the harvesting and processing sectors and the social aspects of fishing communities. This is the proposed role of fisheries management science: the rigorous application of the scientific method of problem solving in the development of strategic alternatives and their evaluation on the basis of objectives that integrate biological, economic, social, operational, and other relevant factors into decision making.

Development of management objectives is a critical element in the success of fisheries management. It is important to define clearly in the context of a structured decision analysis (Healey 1988) the explicit biological and socioeconomic objectives and performance indicators. The approach will undoubtedly vary among species, fisheries, and locations, and will use finer temporal and spatial scales of management than the current approaches.

Viewed as a decision-making problem, fisheries management includes more than fisheries biology considerations generally associated with scientific advice. Decisions should be made on the basis of analytical evaluations and integrated and strategic objectives. The more appropriate term fisheries management advice would be based on a quantitative evaluation of alternatives that would include a projection of their implications on all aspects of the fishery.

These characteristics need to be developed in a framework that uses the tools fundamental to problem solving and decision theory. The suggested basis for this is modelling of the type undertaken in operational research and management science, the field of study that deals formally with problem solving and decision making in organizational systems (Hillier and Lieberman 1974; Lane 1989, 1992*b*). The template from operational research would involve problem formulation, model development, and model verification through experimentation, as well as studies of management structure and industrial organization, so as to provide understandable and useful advice to decision makers (Hillier and Lieberman 1974).

Examples from other areas of resource management, most notably forestry, demonstrate the potential benefits of applied operational research. The model forests project developed in Canada and now being adopted internationally, for example, endeavours to define sustainable development by quantifying and modelling growth, exploitation, and forest use in selected closed areas. These areas are managed and decisions are made in partnership with industrial and community stakeholders (Brand and LeClaire 1994). In fisheries, specific examples of integrated management exist in the Lofoten cod fishery of northern Norway where fishermen have been responsible for managing their own affairs through a legislated system of comanagement with government regulators (Jentoft and Kristoffersen 1989). In Australia, the Australian Fisheries Management Authority acts to coordinate fisheries management decision making through Management Advisory Committees (MACs). The MACs are interdisciplinary committees involving industry and government as partners where government has an advisory role in fisheries management decision making led by industry. These arrangements lead to decision support requirements that demand interdisciplinary modelling and analysis provided through operational research methodologies (Axel and Kaufmann 1995).

Several successful models relevant to decision making in fisheries have been advanced in operational research to date (Lane 1989). The modelling approach, and specifically stochastic models, provide a new foundation for decision making in fisheries management. The inclusion of principles of "management by objectives" (Drucker 1954) for decision making in fisheries management would be a first positive step. These principles include (*i*) the setting of specific goals and milestones, (*ii*) participative decision making and teamwork, and (*iii*) feedback on and appraisal of the performance of goal attainment. The modern philosophy of total quality management (Deming 1982) provides a model procedure for the decision process. Total quality management monitors the effectiveness of decisions toward improving future decisions by continually adjusting the decision and control process on the basis of updated information.

## The evolution of effective fisheries management

The fisheries literature has already begun to reflect recognition of the need for change. However, in our opinion, the few suggestions of modifications to current approaches (Pearse and Walters 1992; Hilborn et al. 1993) do not go far enough, and still retain the gap between biological evaluation and integrated fisheries management.

Hilborn et al. (1993) proposed a two-step process in which a stock assessment group determines biological and economic states and evaluates expected outcomes, but a separate decision-making body examines risks and benefits of possible management actions and formulates a decision. This process offers advantages over the current situation, by linking biological and economic elements and by allowing exploration of a variety of scenarios. However, the process falls short of allowing integrated decisions and the much-needed dialogue among economic and operational interests. The separation of stock assessment and risk assessment prevents the iterative development of the full range of alternatives and assumes that the biological aspects of the problem are insulated from other important considerations including the operational feasibility of decision options, and the anticipated reaction of commercial interests to management decisions. It assumes that the stock assessment group can represent effectively the interests, creative options, reactions, and valuations of the commercial sector by developing feasible biology-based alternatives.

The proposal by Pearse and Walters (1992) that most management decisions can be made by quota holders in a fishery (with safeguards "to protect the broader public interest in resource management") is only feasible within a context that will allow multidisciplinary decision making. Our proposal recognizes the multidisciplinary nature of the problem as having biological (as well as other) constraints and value-based objectives, and suggests that a framework for integrated decision making be developed. Biological, conservation-based constraints, which are of primary importance, may be considered separately (e.g., the evolving debate of what constitutes a safety stock or minimum stock size for conservation of each exploited species, Wooster 1988). However, the full range of management alternatives can only be developed by the decision-making body, taking into account the integrated implications of alternatives. Benefits are derived from sharing information in a structured decision-making framework that defines the contributions of all stakeholders.

Ludwig et al. (1993) pointed out the poor track record in resource management that is caused in part by the mixture of underlying biological and physical complexity, the uniqueness of different situations, and social pressures. They took the rather pessimistic view that complexity will conspire to deflect understanding of the real underlying dynamics, and that consensus will inevitably be in error. Rosenberg et al. (1993), in response to that article, called for "scientific advice based on biological, social and economic considerations" to develop policies that will achieve renewable resource use. We argue that consensus management is possible through implementation of a participatory decision-making structure that demonstrates the relative attributes of various management options or choices. Such an approach would address the major, and legitimate, concerns for the need to include social factors, confront uncertainty, and allow decisions using the best available data at the time. Complete agreement among competing interests in the fishery may not be achieved, but in the process scenarios will have been developed and evaluated so that a decision by a mediating management authority may be based on an analytical evaluation of alternatives, rather than on strictly political concerns. Users with divergent interests will support a process of analytical evaluation of scenarios evaluated against previously agreed to objectives. The result would be a more scientific approach to fisheries management.

Major improvement to fisheries management requires more than a subtle modification of the status quo; it requires conceptual and organizational change. Decisions need to be made on the basis of analytical evaluations and predefined objectives. The claims that sway decisions (from risk of stock collapse to risk of economic loss) should be quantified and demonstrated. Only then can the full impact of a decision be evaluated in the context of all biological, socioeconomic, political, and operational elements. This has not been done to date, in large part because of a lack of a framework for decision making and a lack of specific, appropriate methodology. The concepts outlined in this paper provide the framework for the new discipline of fisheries management science. We propose that further development of this approach through applied case studies on specific fisheries will bring about the evolution required for successful management of fisheries systems, rather than of fish, and will promote the framework and tools required to develop effective fisheries comanagement.

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# Comment—Fisheries Management Science: a plea for conceptual change[1]

**J.F. Caddy**

I find much to sympathize with in the article by Stephenson and Lane (1995), above all with its timing. Fundamentally, I share the authors' perception that this is a time for change, and I believe that our recent apparently pessimistic review of global marine resources (Food and Agriculture Organization 1994) in combination with our diagnostic of global overcapitalization of fleets (Food and Agriculture Organization 1993) provided a major impetus for the current wave of concern on the state of marine resources and their management. However, I am not sure that Stephenson and Lane's diagnosis is necessarily new or complete. A number of authors such as Anderson (1987) have also talked about expanding the fisheries management paradigm. The key question is: Given that we all agree that the paradigm must be changed, what new approach to resource management is appropriate? On this point, however, there are a number of views in circulation.

For the rest of this note I will comment from my personal landscape on the future; without directly contradicting the perspective provided by Stephenson and Lane, I will suggest, however, a difference in emphasis and a number of features not mentioned in their article. First and foremost, I am not sure I fully agree with their diagnostic of the causes for recent management failure. Implicit here seems to be a tendency to blame the development of a flourishing science of population dynamics, predominantly founded in the biological disciplines, for deficiencies in other sectors. They point rightly to a shortage of socioeconomic analysis and its lack of application to resource management, and I do not disagree with the unbalanced nature of current employment in resource biology versus socioeconomics. Since joining the Food and Agriculture Organization (FAO), I have seen how biologists in desperately understaffed institutes in developing countries have to cross disciplinary divides, and the broader holistic perspective this induces. I am happy that scientific bodies such as the International Council for the Exploration of the Sea are now seeing the need for cross-disciplinary linkages with socioeconomics, even if this recognition has been very late in coming in the North Atlantic.

It is difficult for me to see, however, that the blame for the serious stock declines, particularly of demersal resources over the last decade, should be laid at the feet of resource scientists, nor can I see how improved socioeconomic linkages would have done much to prevent stock collapses. Biologists have a defect (if it is one) in fancying themselves spokespeople for the resource, and had they been given more of a say in management decisions, although this alone would not have optimized societal goals for employment and revenue, it would, I am sure, have averted a significant number of stock collapses, and the main socioeconomic options would more likely have been conserved.

So is it simply that the advice of biologists was not heeded? Or was it that the biologists were simply without sufficient influence to effectively demand that current strategies (especially leaky quota controls in an otherwise open access system) should be changed? Was the main sin of assessment scientists to diligently pursue the exhausting annual assessment cycle without raising their perceptions to the heights of "Fisheries Management Science?" Personally, I doubt this diagnosis, if one looks, for example, at the current fashion for risk analysis. Here too, there is an understandable tendency of government scientists to avoid including in their dissection of the uncertainties in fish population parameters the probability of managerial error by the authorities who make the final decisions.

My proposal here (Caddy 1994) was that a watchdog function needs to be incorporated at the highest level in fisheries management structures, similar to that now being installed by merchant banks. Such a system of internal checks and balances is not technique specific and should involve inputs from the wide range of expertise that Stephenson and Lane include in their separately evolving discipline. Perhaps the most fundamental deficiency suggested by the Stephenson and Lane paper can be generalized more widely, namely that the organization of science into disciplines that communicate poorly among themselves and to society does not lead to the necessary synergy or state of precaution in situations of uncertainty. Perhaps the formation of interdisciplinary teams or networks tackling key issues may be one way to go?

My view is that the role and influence of the biologist should be primarily at the limit where the stock becomes endangered. I am not convinced that under these conditions, current economic theory with its emphasis on discounting has much to say, except in suggesting strategies for creating alternative jobs for fishermen in coastal communities, or suggesting strategies to engineer, as painlessly as possible, the reduction of excess capacity from the fishery.

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[1] Paper by R.L. Stephenson and D.E. Lane. 1995. Can. J. Fish. Aquat. Sci. 52: 2051–2056.

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In fact, I suspect that part of the solution to our current overfishing problem will not lie in the biological sector, but in the application of new technologies for improving monitoring and compliance. The context here is provided by the FAO Compliance Agreement, which requires mandatory vessel registries for vessels fishing on high seas areas. Mandatory vessel registration and real effort control, using black boxes to continuously monitor fleet performance, seem to offer a point of departure for an improved information-dense management system, even within national jurisdiction.

The approach FAO suggested at the recent United Nations Conference on Straddling Fish Stocks and Highly Migratory Fish Stocks emphasizied both the use of limit reference points (not all of them biologically based, see, for example, Caddy and Mahon 1995), and a better appreciation of our ignorance and the wide limits of variation in population parameters. A precautionary approach (see, for example, United Nations 1994) is needed. This places the burden of responsibility on harvesters to cut effort promptly once warning bells are sounded or when limit reference points are approached (e.g., reduced spawning stock or effects on associated species), and not on assessment scientists to prove that current levels of effort are not sustainable. If adhered to by decision makers, this precautionary approach would in itself constitute a profound paradigm change.

By the same argument, I do not see that biologists should be given much of a say in setting exploitation objectives as long as the resource is kept in a sustainable condition. In this increasingly rare situation, questions of equity and allocation should predominate over biology, with a dominant role in decision making going to the fishermen. Incidentally, one of the conclusions of FAO (1993), if oversimplified, can be paraphrased as "common property = common poverty." While such a slogan does not provide the full diagnosis, it suggests that one element in the change in paradigm should be to avoid adversarial roles of government and industry, in favour of enlightened self-interest by an industry that is convinced that short-term interests should not predominate over long-term objectives.

A key role that biologists should be playing over the next two decades in conjunction with socioeconomists is to develop realistic strategies for restoring stocks and habitats to their once-productive state. A further role is to monitor environmental degradations (as in the Black and Baltic seas) and regime shifts such as those that are linked to climatic and anthropogenically caused changes. Here the real need for another paradigm shift away from equilibrium-based thinking can be linked to growing evidence that decadal-scale changes are occurring (e.g., in many fisheries of the North Pacific), not all of which are to be laid at the door of fisheries managers.

One way that FAO is attempting to address the management problem is in developing a code of conduct for responsible fisheries, which, although voluntary, once adhered to by coastal states, will provide a touchstone and formal set of criteria in the public sector against which management decisions can be judged. Such a code of conduct may be a unifying force in ensuring that all concerned have foremost in their thoughts the need to ensure continuity of the resource.

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# Comment—Fisheries management science: a plea for conceptual change[1]

**R.W. Gauldie**

Stephenson and Lane (1995) wrote, in proposing a new fisheries management science, of "the emphasis on socioeconomics integrated with ecology" to overcome the shortcomings of current fisheries management systems. I cannot comment on socioeconomics, having never been in either politics or business, but I can comment on ecology: there simply isn't any. There is ecology in the sense of post facto statistical correlations among the natural history data that we have spent billions of taxpayers' dollars to collect, but we do not have any satisfactory scientific theories (which is what I wish ecology really constituted) that explain the variation in abundance of a single plant or animal in the scientific sense of predicting future changes in abundance (Krebs 1985, 1991). It is at the level of an explanatory theory of ecology where I think that the problem of fisheries management lies. My argument is that governments have claimed the right to manage fisheries by regulation because they have scientific models that can alleviate risk to both the capital invested by fishermen and the social capital invested in communities that are dependent on fish, by accurately predicting changes in the abundance of fish. There is an implied social contract of long standing: the fishermen gave up their property rights over the fish to the government, in return for the government protecting their invested capital by managing the fishery to produce a safe, sustainable, maximum yield. That government fisheries management agencies have failed in their part of the contract to provide an accurate prediction of abundance has been clear for many years, as Stephenson and Lane point out, and yet the social contract remains so strongly enforced that the fishermen continue to be held to the losing end of the deal. How did such a situation ever come about?

A brief synopsis of the history of the fisheries management helps to see the problem more clearly and points to the answer. One of the first significant sums of money spent on research into fish abundance was 200 pounds sterling given to Prof. M'Intosh of St. Andrews University to look into the effects of steam trawlers on fish stocks on the northeast coast of Great Britain. M'Intosh's report was attached to the *Blue Book* produced by the British Parliamentary Commissioner on Fisheries in 1885 (Anonymous 1885).[2] The *Blue Book* contained all the elements of the social contract that was to be developed over the next century, namely an invitation by the fishermen to the government to intervene (there were riots and threats of murder against steam trawlermen unless the government intervened) not only to guarantee the supply of fish but also to defend some vaguely defined but highly desirable social values; for the noble class of artisanal fishermen in 1885 (Anonymous 1885) one might read the noble ideals of the Green movement in 1995.

Government intervention in fisheries management increased rapidly after 1885. By 1901 the Council for the Exploration of the Sea had been founded to coordinate the intergovernment regulatory agencies in Europe that were controlling fishing in the North Sea, Baltic Sea, and North Atlantic. What would eventually be the biggest boost to government control came when Baranov (1918) developed his model of scientific management of fisheries. Although largely dialectical in origin, Baranov's model of a scientifically controlled, beneficial interaction between fish and fishermen eventually led to the quantitative treatment of fisheries by Beverton and Holt (1957).[3] Beverton and Holt

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[1] Paper by R.L. Stephenson and D.E. Lane. 1995. Can. J. Fish. Aquat. Sci. 52: 2051–2056.

[2] This editorial in Blackwoods is particularly informative on the political developments in fisheries in Great Britain from 1862 to 1885 that resulted from the deployment of steam trawlers. All of the issues with which we are today familiar, decline in abundance, damage to habitat, socioeconomic dislocation, fragmented scientific research efforts, and mixed political responses, are lucidly discussed. There was, in addition, considerable sympathy for the fishermen at that time: "the seductive phrases, 'food of the people,' 'progress,' and 'free trade,' fail to make up for the extinction of such a class as the fishermen of our village ports. It is not merely that such men are of immense importance to a maritime nation as a nursery for seamen, and as the first line of our naval reserve; but in this time of luxury and domestic peace, the fishermen are almost the only class who keep up something of the courage and simplicity of older times.... For such a class to disappear is, in our opinion, a national calamity."

[3] On page 328 Beverton and Holt wrote: "...Baranov arrived at what we believe to be a correct appreciation of the behaviour of an exploited fish stock from a study of his theoretical model..." Accompanying this comment, Beverton and Holt's figure 17.29 shows the increase in yield per gram-recruit proposed by Baranov as a response to fishing pressure.

downplayed the scientific side of Baranov's dialectical approach. Today, we don't think of Marxist dialectic as science, but at the time, and in the sense of supplying a fundamental and universal underlying principle, Baranov's approach was scientific in spirit if nothing else. By the time of Beverton and Holt, the dialectical approach had disappeared; the idea of increasing catch rates by removing older fish (the synthesis of the dialectic) had never worked in practice. Beverton and Holt appealed to the von Bertalannfy growth curve as the scientific rationale for some of their treatment, but generally their approach was in the form of a mathematical model simulating the effects of fishing. The various terms of the model were given biologically evocative, scientific-sounding names but essentially they were ad hoc statistical summaries of the data. The mathematical simulation model approach of Beverton and Holt was not successful, but it led to a succession of equally unsuccessful models in which even the limited scientific principles contained in Beverton and Holt's model were discarded, eventually leading to the modern almost entirely mathematical treatment that has become in essence an extremely well-crafted statistical time series. The difference between scientific theory and statistical time series can be seen in the response by Rothschild (1975) to Radovich's (1975) complaint:

"The real test of a model, of course, is how well it predicts, not whether or not we use biological sounding terms to describe its coefficients and exponents. If it doesn't work, something is wrong with either the mathematics, the assumptions, or the data. Population dynamicists usually blame the data, or occasionally the animal itself, for not behaving in a predictable manner."

To which Rothschild replied:

"My first point is that the problem of deviation from models is an indication that models perhaps are not quite what they might be. I think this is probably not the most important function of models, but the most important function of models is to generate questions. If you have a model, it means you are modeling through a problem rather than muddling through a problem and you can therefore ask questions, 'Where have I gone wrong?' "

Rothschild's comment warrants close attention: mathematical models are not scientific theories. One cannot ask mathematical models to provide the kind of predictive output characteristic of scientific theories. However, scientists in government fisheries management agencies have been driven by decreasing funding and increasing responsibilities for more, new fisheries to rely more and more on mathematical models. It is an invidious position to be charged with solving politically created problems while being denied by politicians the resources to do so.

Although the rationale behind government regulation of fishing effort was based on the government's ability to alleviate risk by scientific and mathematical models, the regulations themselves were never contingent on the success of the models. Regulations were introduced on the assumption that the models would eventually work. By the time that it was realized that the models would not work, the regulations were firmly in place. The idea of an exchange implied in the social contract was gone. The loss of fishermen's rights were no longer compensated by the government's ability to alleviate risk. Government agencies simply assumed that it was the government's right to regulate the property rights of fishermen and give nothing but regulated effort in return. The bait and switch was complete. The government now often appears to have the view that fishermen are privileged by even being allowed to go fishing.

Clearly, government agencies can no longer claim to be able to alleviate risk to the invested capital of the fishermen by using scientific or mathematical models to predict abundance. Governments have been managing fisheries by regulation, not by their proven ability to alleviate risk. It is very important that we return as quickly as possible to the focus of alleviating risk. Throughout the brief history of managed fisheries the alleviation of risk to invested capital has been the fundamental rationale of scientific fisheries management.

I believe that Stephenson and Lane are quite correct in their proposal to introduce business methods to the problems of fisheries management; however, it must be made clear that those problems center on the risks to both fishermen's capital and to social capital caused by the present unpredictability of changes in fish abundance. The problem does not lie in the regulations per se. Better drafted, more goal-directed, more objective regulations are unlikely by themselves to overcome the problem of not being able to predict fish abundance: we have a hundred years of experience in writing unsuccessful regulations as proof of the failure of regulations. The tools of business should be applied to what is a business problem. This does not mean an MBA in each regional fisheries laboratory, and a half-dozen more at head office. It means handing over to the fishing industry the responsibility and cost for managing their own risk. It should not be a government responsibility to pay for the management of profitability of the fishing industry by managing their risk. Every business has to overcome the risk inherent in cycles in abundance: abundance of customers, abundance of raw materials, abundance of finance, etc. The tools that other business enterprises use are available to the fishing industry to manage their own risks and they should be left to do so.

However, what will happen to the thriving cottage industry of regulation writers and mathematical modelers who have supplied what little substance there has been to government regulation? What will happen to those universities that have built schools of fishery science around the demand for fisheries mathematical modelers and biologist technicians? I believe that the long-term answer lies in allowing the fishing industry to employ those scientists who can make a contribution to risk management, and allowing the universities to return to the still-unsolved problem of producing ecological theories that are as effective in prediction as theories are in biochemistry and physiology. Maximum sustainable yield should not be an issue for government intervention. The issue of interest to the public good that requires continuing government and fishing industry support is something closer to minimum sustainable population. Farmers can do as they like to their land, but we do not allow them to destroy it, and we use their, and our, tax dollars to support their wise utilization of their own land. Similarly, we should focus on preserving the minimum populations of fishes that in their years of plenty will provide food for the table and profit for the industry. We should also recognize that our enjoyment of the fish of the sea is not confined to eating them. Governments have a responsibility to spend taxpayer's money to set aside the fish resources to which some of us feel strong emotional ties.

These are big enough problems to keep us all occupied for a long time, and problems to which Stephenson and Lane's approach could be fruitfully applied. Within fisheries research funding agencies (both in industry and government) we need to identify success by more businesslike measures than consensus. We need scientific managers with clear goals and the authority to spend money to reach those goals. We need to bring the peer review system back into perspective by making managers, not anonymous peer review committees, responsible for, and rewarded by, their own success or failure. In terms of reorganization of effort we certainly need management by objectives, of which the first objective is a rational scientific (not a mathematic or some other kind of metaphysic) theory of changes in fish abundance. But the problem of a theory of abundance is still the biologists' to solve. Unless we achieve this first objective then fisheries management science will soon join all of the other withering fig leaves that we have used to hide our shameful ignorance of the ecology of fish.

## Acknowledgments

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ERRATUM

# Erratum: Stock-recruitment models for Pacific sardine (*Sardinops sagax*)

**Larry D. Jacobson and Alec D. MacCall**

**Ref.**: Can. J. Fish. Aquat. Sci. 52: 566–577 (1995).

The NRC Research Press regrets that some of the values in Table 1 were shifted during the production process. The table has been reprinted correctly below.

**Table 1.** Spawning biomass, recruitment, and sea surface temperature (SST) data for sardine by fishing season.

| Fishing season | Spawning biomass ($10^3$ t) | No. of recruits ($10^6$ fish) | Mean three season SST (°C) | Mean five season SST (°C) |
|---|---|---|---|---|
| 1935 | 3526 | 4 098 | 16.56 | 16.78 |
| 1936 | 3417 | 2 821 | 16.84 | 17.02 |
| 1937 | 3628 | 5 383 | 17.11 | 16.96 |
| 1938 | 2847 | 6 940 | 17.12 | 17.14 |
| 1939 | 1689 | 6 763 | 16.98 | 17.30 |
| 1940 | 1207 | 11 808 | 17.15 | 17.20 |
| 1941 | 1202 | 14 442 | 17.31 | 17.19 |
| 1942 | 1609 | 6 152 | 17.50 | 17.28 |
| 1943 | 1761 | 3 268 | 17.21 | 16.94 |
| 1944 | 2459 | 3 720 | 16.94 | 16.79 |
| 1945 | 2066 | 2 385 | 16.72 | 16.81 |
| 1946 | 1679 | 1 625 | 16.64 | 16.67 |
| 1947 | 1261 | 1 667 | 16.72 | 16.54 |
| 1948 | 720 | 3 875 | 16.76 | 16.57 |
| 1949 | 566 | 4 261 | 16.51 | 16.53 |
| 1950 | 405 | 3 690 | 16.31 | 16.43 |
| 1951 | 740 | 290 | 16.41 | 16.37 |
| 1952 | 793 | 397 | 16.55 | 16.46 |
| 1953 | 780 | 972 | 16.43 | 16.49 |
| 1954 | 277 | 1 197 | 16.39 | 16.42 |
| 1955 | 136 | 382 | 16.41 | 16.52 |
| 1956 | 202 | 264 | 16.50 | 16.86 |
| 1957 | 239 | 588 | 16.65 | 17.15 |
| 1958 | 170 | 1 586 | 17.04 | 17.35 |
| 1959 | 108 | 905 | 17.62 | 17.39 |
| 1960 | 90 | 288 | 17.80 | 17.23 |
| 1961 | 177 | 111 | 17.39 | 16.99 |
| 1962 | 122 | 74 | 16.77 | 16.74 |
| 1963 | 88 | 56 | 16.43 | 16.51 |
| 1985 | | (131) | 17.80 | 17.76 |
| 1986 | 5 | 88 (85) | 17.87 | 17.68 |
| 1987 | 18 | 57 (317) | 17.71 | 17.44 |
| 1988 | 24 | 212 (240) | 17.54 | 17.39 |
| 1989 | 33 | 161 (355) | 17.23 | 17.37 |
| 1990 | 56 | 238 | 17.18 | 17.39 |

**Note**: Historical spawning biomass for 1935–1963 is sardine age 2 and older. Recent spawning biomass for 1985–1990 is sardine age 1 and older. Reliable spawning biomass and recruitment estimates for sardine during 1964–1984 are not available (Barnes et al. 1992). Recruitment is the number of age 2 fish (millions) on July 1 of the fishing season indicated. Recent recruitment estimates derived from original data as described in the text (original data in parentheses). For example, 5 000 t of sardine spawned during 1983 and produced 131 million age 1 sardine that recruited on 1 July 1984 or equivalently as 131 × 0.67 = 88 million 2-year-olds on 1 July 1986.

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1. *Faire une revue* de l’état actuel des connaissances théoriques et pratiques de la zone côtière, incluant les aspects scientifiques, économiques et sociaux et de l’évolution de celle-ci.
2. *Fournir un forum* en permettant un rassemblement d’intervenants de différents milieux gouvernementaux et universitaires, de l’industrie et d’autres groupes intéressés à l’aménagement, le développement et l’utilisation de la zone côtière.
3. *Émettre des recommandations* à partir des délibérations de la Conférence ZCC ‘96 afin que les gouvernements puissent les intégrer dans leurs stratégies de gestion intégrée et de développement durable en ce qui a trait aux zones côtières sous leurs juridictions. Établir de nouvelles directions de recherche.

Nous sollicitons des résumés et études de cas portant sur les sujets à caractère national ou international et relatifs à la zone côtière ou à l’océan qui sont énumérés ci-après, ainsi que sur d’autres sujets traitant de la zone côtière et de l’océan. Les propositions de sujets de tables rondes sont aussi les bienvenues.

- Outils scientifiques de contrôle, de classification, de surveillance et de gestion des zones côtières;
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- Considérations culturelles, socio-économiques et politiques;
- Questions juridiques et résolution des conflits;
- Développement, conservation et protection de la zone côtière et ses ressources;
- Collectivités côtières : survie et développement;
- Éducation et formation;
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- Agenda 21 : le temps d’agir.

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Pour obtenir d’autres informations concernant cet événement, vous devez écrire au **président de la conférence M. Mohammed El-Sabh, Groupe de recherche en environnement côtier (GREC), 310 allée des Ursulines, Rimouski (Québec), Canada G5L 3A1. Tél.: (418) 724-1707; télécopieur: (418) 724-1842; Courrier électronique: mohammed_el_sabh@uqar.uquebec.ca.**

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1. *To review the development of knowledge* - both theoretical and practical - of the coastal zones, including the scientific, managerial, social and economic aspects, since the CZC '94 Conference held in Halifax in September 1994.
2. *To achieve this review* by bringing together representatives of governmental, academic, business and coastal communities and other interest groups involved in the management, development and use of coastal zones.
3. *To frame recommendations* arising from the deliberations of the conference and to outline new research and management directions. Such advice will be forward to Governments to contribute to the formation of strategies for the integrated management and sustainable developments of coastal zones under their national jurisdiction.

*Papers and case study presentations* are invited from national and international coastal zone stakeholders, community-based organizations, scientists and engineers, governments and primary resource users, industry and business. This international conference will feature oral and poster presentations, plenary panel sessions and round-table discussions in the following subject areas:

- Scientific Tools for Monitoring, Classification, Surveillance and Management of Coastal Environ-ments
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- Legal Issues and Problem Solving in a Multi-Use Environment
- Conservation and Protection of the Coastal Zone
- Survival and Development of Coastal Communities
- Public and Formal Education in Coastal Zone Management
- Promotion of Environmentally Sound Technology
- Management and Development of Coastal Zone Resources
- Coastal Zone Policy and Institutional Arrangements
- Regional and International Issues
- Agenda 21 : A Vehicle for Action?

The CZC '96 Conference will be held at the Université du Québec à Rimouski Campus and is organized by the Groupe de Recherche en Environnement Côtier (GREC), Université du Québec, jointly with the Coastal Zone Canada Association and Fisheries and Oceans Canada.

For more information or in order to receive the second announcement, please write to the **Conference Chair, Professor Mohammed El-Sabh, Groupe de recherche en environnement côtier (GREC), Université du Québec, 310 allée des Ursulines, Rimouski, Québec, Canada G5L 3A1. Tel.: (418) 724-1707; fax : (418) 724-1842; Internet : mohamed_el_sabh@uqar.uquebec.ca.**

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